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STUDIES OF POLYOMA VIRUS

Pathology And Distribution of Tumors Produced in Syrian Hamsters Following Intracranial or Subcutaneous Inoculation[1]

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INTRODUCTION

Polyoma virus produces disseminated neoplasms in mice, rats, hamsters and rabbits (1, 2, 3, 4). Preliminary studies of tumor production with polyoma virus in hamsters have shown that tumors frequently occur at the site of injection of virus (5). The question arose, therefore, whether intracerebral tumors could be induced by this virus. Experiments were designed to investigate this possibility, to study further the problem of tumor localization, and to compare the histology of tumors occurring at the site of injection with that of tumors arising elsewhere in the body.

MATERIALS AND METHODS

Virus. The polyoma virus used in these studies was concentrated and partially purified from infectious mouse embryo tissue culture fluids as previously described (6). Briefly, these procedures entailed precipitation of the virus at pH 4 with 15 per cent methanol, resuspension of the virus at pH 8.5 with 0.88 M NaCl, and two cycles of ultracentrifugation. The resuspended virus was passed through an ultrafine sintered glass filter. Infectivity assays were made in mouse embryo tissue culture as described elsewhere (6). Prior to use, the virus was diluted to give $10^{5.5}$ mouse embryo tissue culture doses ($TCID_{50}$) per 0.05 ml.

Injection of Hamsters with Virus. Pregnant hamsters were obtained from Lakeview Farms 7 to 10 days prior to term. Each litter usually consisted of 8 to 14 hamsters which were injected with virus within several hours after birth. A volume of 0.03 ml. was delivered from a 0.25 ml. syringe tipped with a half-inch 30 gauge needle. For intracerebral injections the needle was inserted to a depth of 3 mm. at a point 2 mm. to the left of the sagittal suture and 2 mm. anterior to the lambdoid suture. Subcutaneous injections were made in the interscapular region. To minimize the effects of variation from litter to litter, some litter mates were injected with virus intracerebrally and others

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subcutaneously. Each litter also contained animals injected with sterile diluent fluids by either route.

Following inoculation, the hamsters were returned to their mothers. At 21 days of age they were placed in individual cages. The animals were inspected daily and those showing signs of impending death were killed with ether. All survivors were killed at 90 days of age. Autopsies were performed on all animals.

Tissue Culture of Hamster Tumor. A subcutaneous tumor, (017-1) which developed in a hamster following injection of 10^5 $TCID_{50}$ of polyoma virus, was excised, trypsinized, and the cells were cultivated *in vitro* in monolayer cultures as previously described (5). Extensive tests of this cell line established that it lacked demonstrable virus (5).

After 214 days of cultivation approximately 10,000,000 cells were scraped from one 32 oz. culture bottle into the tissue culture fluid. The suspended cells were then centrifuged at 1000 r.p.m. for 2 minutes. All but 2 ml. of the tissue culture fluid was withdrawn. The cells were resuspended in the remaining fluid.

Injection of Hamsters with Tumor Cells Cultivated in Vitro. Newborn hamsters were injected subcutaneously and intracerebrally with resuspended tumor cells. The technique of injection and the subsequent handling of hamsters was the same as described for animals injected with virus.

Histologic Processing. Organs and subcutaneous tumors were fixed in 10 per cent formol-saline. Most brains with externally visible tumors were embedded uncut. The remainder were sectioned coronally into four blocks. The blocks of brains and other organs were embedded in celloidin or paraffin.

All brains in which tumor was detected on gross examination were sectioned serially at 8 to 15 microns. Sections were stained with hematoxylin and eosin, Mallory's trichrome, Masson's trichrome, van Gieson's stain for collagen, Perdrau's stain for reticulum, Greenfield's phosphotungstic acid hematoxylin and cresyl violet.

EXPERIMENTAL RESULTS

A. Detection, Incidence and Location of Tumors Induced with Polyoma Virus

1. Detection of Tumors

Subcutaneous tumors were readily detected by visual inspection. The presence of internal tumors was often manifested by such external signs as inactivity, lethargy, pallor of the mucous membranes, rapid breathing, epistaxis, weakness, paralysis in fore or hind limbs, head torsion and disequilibrium. On the other hand many hamsters with tumors died without external signs of disease.

At autopsy, examination of the organs led to the detection of all animals with tumors. Tumors in liver, lungs, and heart were most easily recognized grossly. However, in 50 per cent of the kidneys and 25 per cent of the brains containing tumors, these were detected only by microscopic examination. The incidence of tumors as found in different organs by gross and microscopic examination is shown in Table I.

It is evident from the foregoing that the tumor incidence listed in Tables

TABLE I

Detection by Gross and Microscopic Examination of Tumors Induced by Polyoma Virus

Site of tumor	No. of animals with tumors	No. of animals in which tumor was detected by gross examination	No. of animals in which tumor was detected only after microscopic examination
Brain	42	29	13
Subcutaneous	18	18	0
Liver	46	46	0
Lung	47	47	0
Heart	42	39	3
Kidney	17	8	9

TABLE II

Anatomical Location of Tumors Induced by Polyoma Virus

Location of Tumors	Animals with tumors after intracerebral injection of virus		Animals with tumors after subcutaneous injection of virus	
	No.	%	No.	%
Intracranial	42	70	0	0
Subcutaneous				
Under the scalp	7	12	0	0
Interscapular region	0	0	11	50
Other sites	0	0	0	0
Heart	33	55	9	41
Liver	37	60	9	41
Lungs	38	63	9	41
Kidneys	12	20	5	22

I, II, and III refers to minimal values. It is probable that more extensive examination of organs by serial sectioning would have yielded additional microscopic lesions.

2. Incidence of Tumors and Mortality of Hamsters

Of the 60 animals injected intracerebrally, 58 developed tumors. Of this number, 50 hamsters died before 90 days of age. The remaining 8 were found to have tumors when killed at 90 days of age. Of the 22 animals injected subcutaneously, 17 developed tumors. Of this number 14 died before 90 days, and 3 had tumors demonstrated when killed. As shown in Figure 1 the cumulative mortality rate of hamsters injected with polyoma virus intracerebrally exceeded that of animals injected subcutaneously. Very likely the difference in mortality rates was due to the fact that intracranial tumors need not grow as large as some of those formed elsewhere to cause the death of the animal.

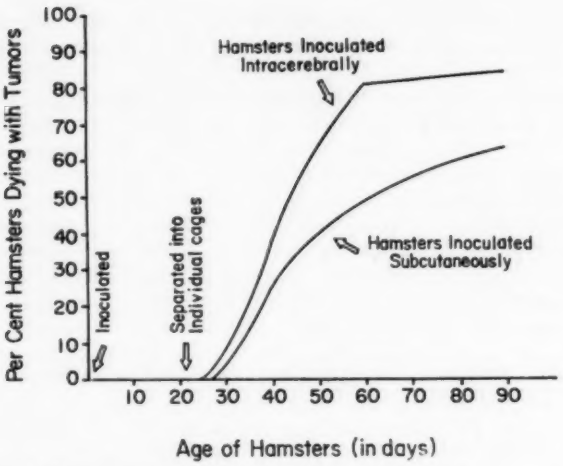

FIG. 1. Cumulative percentage of animals dying with tumors.

All animals injected intracerebrally or subcutaneously with sterile diluent fluids were found free of tumors when killed at 90 days of age.

3. Location of Tumors

The anatomical location of tumors in hamsters is shown in Table II. It is clear that there was specific localization of tumors at the site of virus injection. Intracranial tumors developed in 42 (70%) hamsters injected intracerebrally but in none of those injected subcutaneously. Subcutaneous tumors developed in the interscapular region in 11 (50%) hamsters injected subcutaneously but in none of those injected intracerebrally. In 17 (12%) animals injected intracerebrally subcutaneous tumors developed under the scalp, at the site of injection. The incidence of tumors in heart, lung, liver and kidney, in animals injected by either route, was of the same order of magnitude.

The location of tumors with reference to the site of injection is shown in Table III. The greatest number of hamsters developed tumors both at the site of injection and elsewhere. Some hamsters developed tumors only in organs distant from the site of injection. A few hamsters showed tumors solely at the site of injection. Finally, no tumors were found in some animals.

B. Pathological Findings in Hamsters Injected with Virus

1. Gross

Subcutaneous tissue: Localized subcutaneous tumors developed only at the sites of injection: in the interscapular region, or overlying the calva-

TABLE III

Location of Tumors with Reference to the Site of Injection of Virus

Location of tumor	No. of animals with tumors after injection of virus	
	intracerebrally	subcutaneously
Site of injection only		
Intracranial	3	0
Intracranial & subcutaneous	1	0
Subcutaneous	2	4
Site of injection and elsewhere	38	7
Distant from site of injection only	14	6
No tumors	2	5
Total	60	22

rium. The tumors appeared as single or multiple nodules, irregular in shape, and varying in size from 0.5 to 5 cm. They were freely movable in the subcutaneous tissue, and were not adherent to skin, muscle or bone. The small nodules were firm in consistency and pearly white. The larger tumor masses were soft, and on section showed areas of hemorrhage.

Heart: In the heart, tumors were located in the walls of both atria and ventricles, but most often in the right atrium. Grossly, they could be seen as white spheroidal masses which usually projected into the lumen of the chamber. In most hearts, there were multiple tumor nodules, measuring 1 to 2 mm. in diameter (Fig. 2A). Occasionally in a heart containing only one nodule in the atrium, the mass reached a diameter of 20 mm. filling most of the thoracic cavity. Such large tumors usually showed irregular areas of softening and hemorrhage.

Lungs: The most frequent abnormality in the lung consisted of multiple dark red spherical masses, less than 0.5 mm. in diameter, scattered throughout the organ (Fig. 2B). In an occasional animal, in addition to these lesions, there was massive hemorrhage in one or both lungs. In one animal there were two dark red spherical nodules, 1 mm. in diameter, as well as several pearly white nodules of the same size. The lungs of another animal contained only multiple white nodules.

Liver: Single or multiple blood-filled bulbous masses, 1 to 5 mm. in diameter, projected from the surfaces of the liver, most frequently from its undersurface and edges. On cross section, these lesions were found to extend deeply into the parenchyma of the liver and on occasion to contain an irregularly shaped white nodule (Fig. 2C). Often such a lesion ruptured, and resulted in massive intraperitoneal bleeding.

Kidneys: Unilateral, or less frequently, bilateral tumors in the kidneys usually appeared as single, 1 mm. spherical white nodules at the cortico-

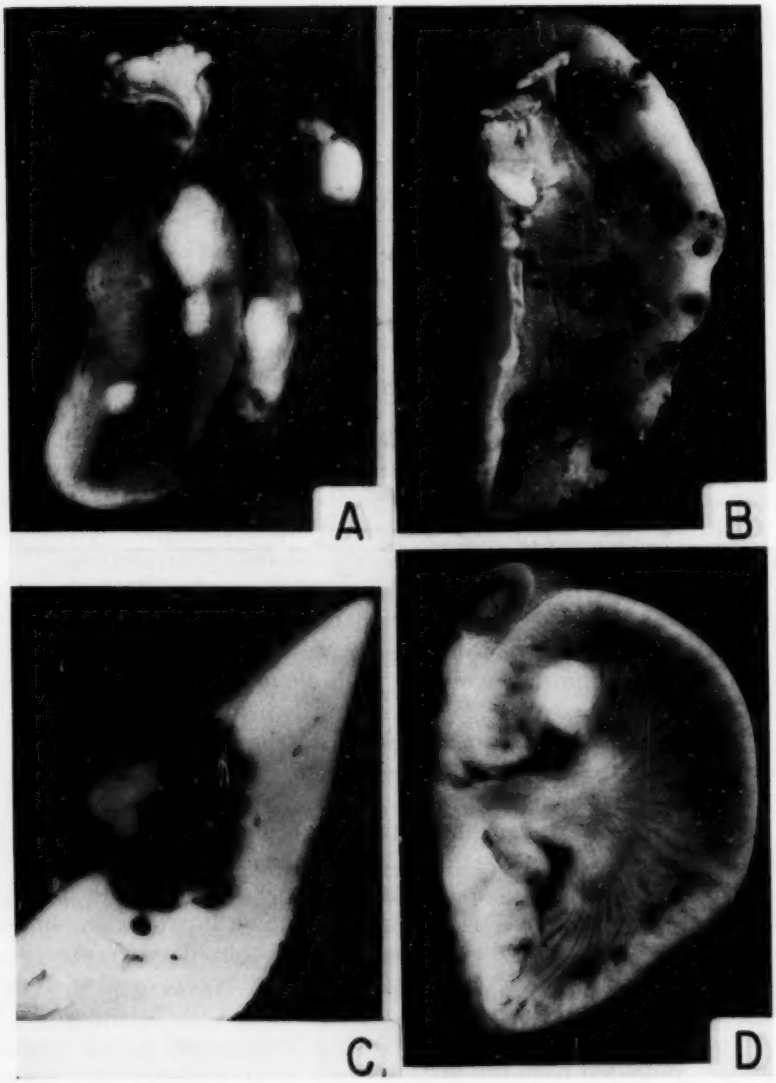

FIG. 2. A. Multiple tumors in heart. B. Hemorrhagic tumor nodules in lung. C. Hemorrhagic tumor in liver. D. Tumor nodule in kidney.

medullary junction (Fig. 2D). Occasionally, multiple nodules of nearly equal size occurred in one kidney. Rarely a large tumor mass 3 to 4 mm. distended the kidney. In one animal, two tumors were found in the kidney pelvis.

Brain: Gross examination of the brains revealed several patterns of tumor growth.

The most common finding on examination of the convex aspect of the cerebral hemispheres was the obscuration of the interhemispheric fissure over most or all of its length (Fig. 3A). On coronal section, pearly white, firm tumor tissue was found to distend the fissure, compressing and distorting the diencephalon and the cerebral hemispheres (Fig. 4A). Serial sections showed that the tumor tissue occupied a considerable portion or the entire length of the fissure. In a few brains anatomical details in the region of the lateral sinus were also obscured by tumor.

In addition to, or in the absence of, findings described above, on the external surface of some brains there were one or several white circular areas firm to palpation (Fig. 3A, B). These were surrounded by moderately large blood vessels. Such lesions were usually seen on the surface of the cerebral hemispheres, and only rarely on the surface of cerebellum or brain stem. In coronal sections, some of these tumors appeared lenticular in shape; others occupied most of the cerebral hemisphere.

In seven animals, white firm spheroidal masses, 1 to 4 mm. in diameter were found in one cerebellopontine angle (Fig. 3C). Such masses produced gross deformity of brain stem, cerebellum and occipital lobe. Tumors in this location were adherent to the dura mater and could be easily shelled out from the deformed brain (Fig. 3D).

Moderate or marked hydrocephalus was seen in several brains containing tumor, as well as in some brains in which no tumor was visible grossly.

Peripheral Nerves: In one animal with a forelimb paralysis, the brachial plexus was compressed by a firm paraspinal tumor. In another animal with a hind limb paralysis, the lumbosacral plexus was embedded in a retroperitoneal tumor mass.

2. *Microscopic*

All tumors were sarcomas. The solid tumors varied in morphology between two extremes. One extreme was represented by tumors which were densely cellular (Fig. 5A). Their cells were arranged in random fashion, had scant cytoplasm, and contained round or oval nuclei. Such tumors had a moderate density of reticulum fibers (Fig. 5B) and little or no collagen. Tumors of this variety were encountered most frequently in kidneys and lungs. The other extreme was represented by less cellular tumors with elongated cells arranged parallel to each other in interlacing streams (Fig. 5C). Such tumors had a rich network of reticulum fibers (Fig. 5A) and abundant collagen. This type of tumor was most frequently found in the subcutaneous tissue, heart and brain (Fig. 6A). Two of the tumors located in the cerebellopontine angle and one tumor in the atrium of the heart had areas of osteoid and bone formation.

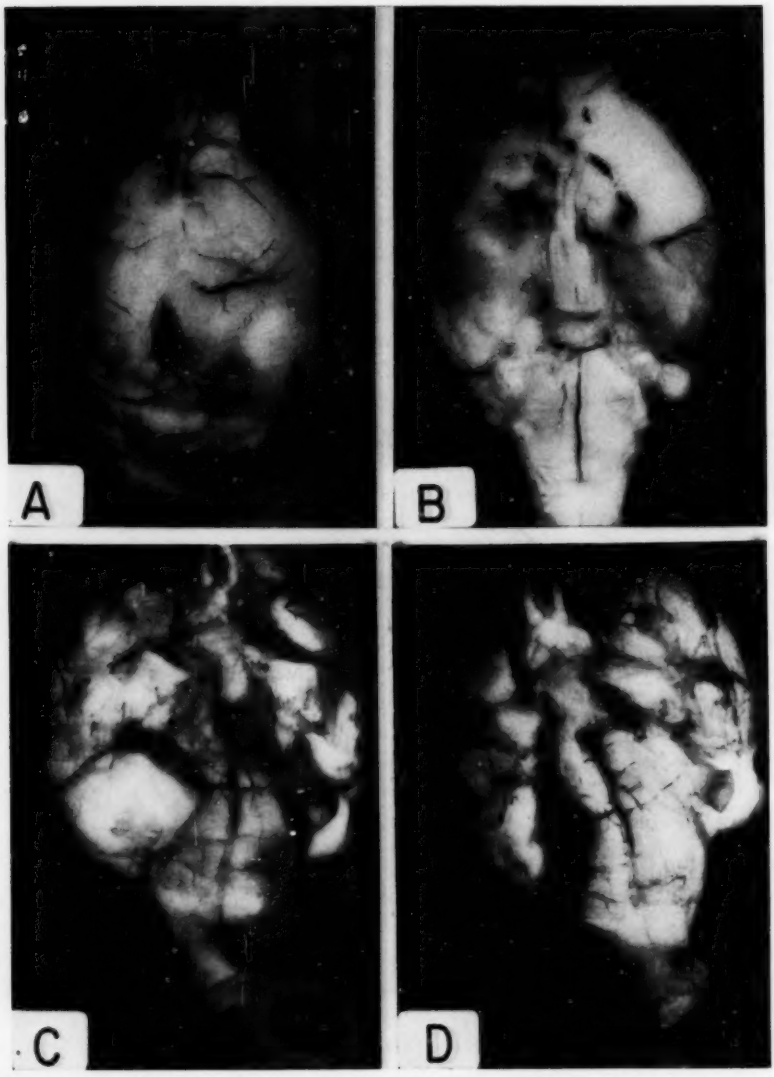

FIG. 3. A. Dorsal view of brain showing partial obliteration of interhemispheric fissure by tumor and a tumor nodule in the right parieto-occipital region. B. Ventral view of brain showing extensive tumor over the anterior half of the left cerebral hemisphere. Large blood vessels are visible at the edge of the tumor. C. Ventral view showing a large tumor in the cerebello-pontine angle. D. Same as 2C with the tumor shelled out to show the deformed brain.

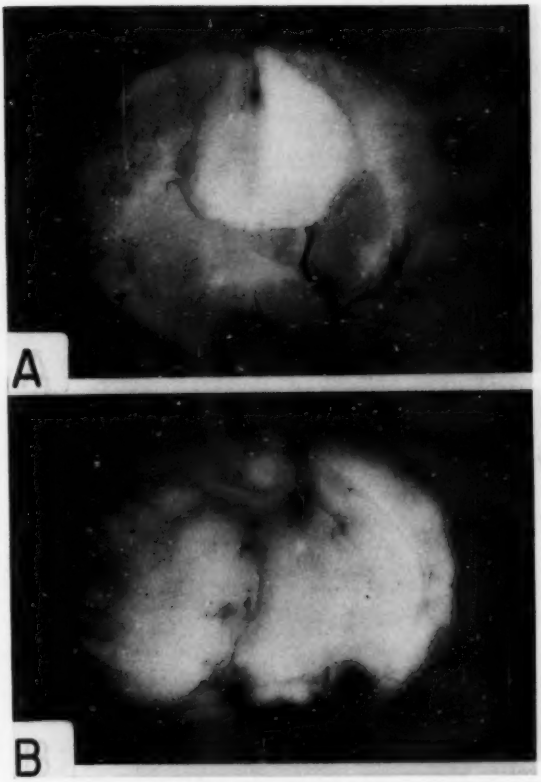

FIG. 4. A. Coronal section of brain showing massive tumor growth in the interhemispheric fissure. B. Coronal section of brain showing large tumor mass and small nodule in the left cerebral hemisphere.

The cell nuclei in all these tumors showed considerable variation in size, shape and chromatin content. One subcutaneous tumor, in addition to its moderately sized cells, contained a number of very large cells with comparably large single nuclei. Inclusion bodies were not seen. Mitotic cells were abundant. In general, the tumors contained many thin-walled blood vessels. The large-sized tumors frequently showed areas of hemorrhagic necrosis. A true capsule was never present. The degree of invasiveness, however, varied from one tumor to another. At the periphery of most tumors, neoplastic cells had infiltrated among the normal cellular elements of the organ.

In the majority of brains there was extensive growth of tumor in the leptomeninges (Fig. 7A). Such growths usually covered wide areas of brain

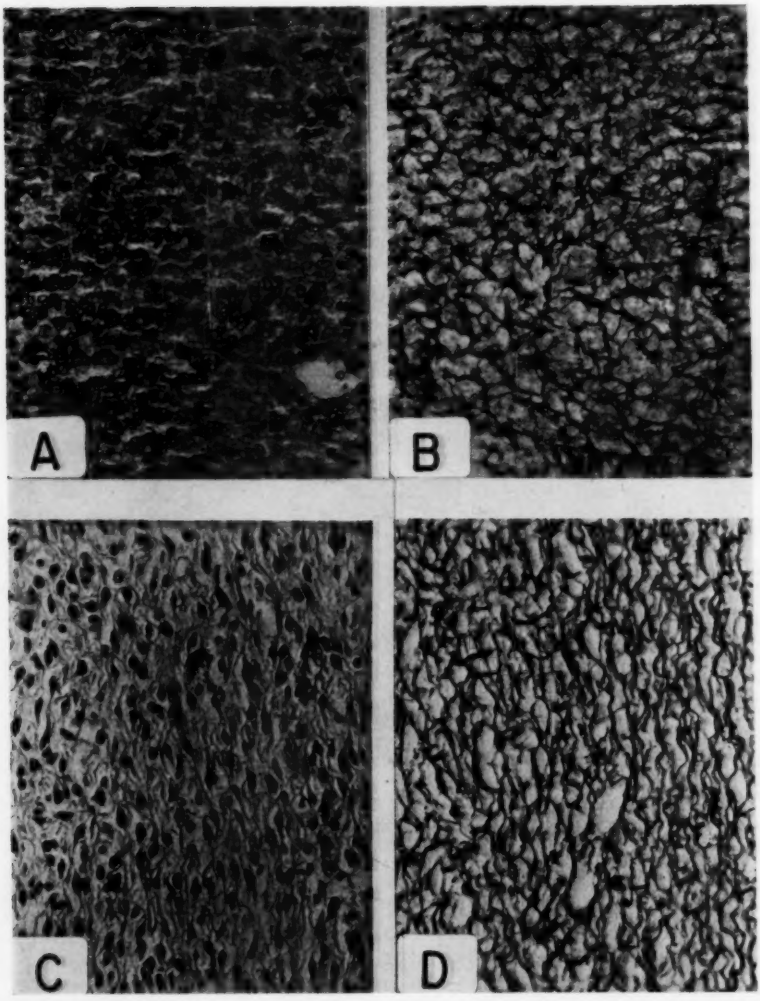

FIG. 5. A. Densely cellular tumor in lung. H & E × 400. B. Same as A. Perdrau stain for reticulum fibers × 400. C. Subcutaneous tumor. H & E × 400. Same as C. Perdrau stain for reticulum fibers × 400.

and were continuous with large spheroidal or cylindrical masses of tumor growing in the interhemispheric fissure, or in the region of the tentorium. The tumors invaded the brain substance most often in the form of perivascular cords and only rarely by diffuse growth (Fig. 6B). Large tumor

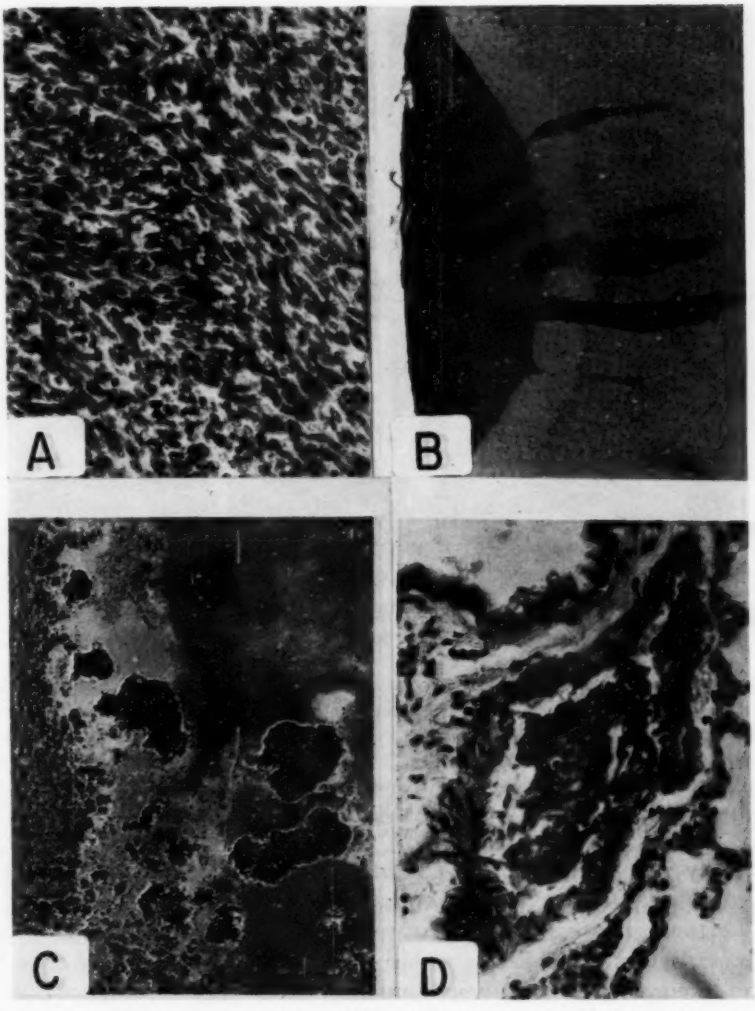

FIG. 6. A. Tumor in brain. H & E × 400. B. Perivascular growth of tumor into brain from leptomeninges. H & E × 80. C. Clusters of anaplastic tumor cells inside hemorrhagic lesion in liver. H & E × 80. D. Intravascular growth of tumor cells in lung. H & E × 400.

masses located inside the brain were usually connected, by means of perivascular cords, with the tumor in leptomeninges. Often, the tumors compressed or invaded the third or fourth ventricle and caused hydrocephalus by obstructing the flow of cerebrospinal fluid (Fig. 7C, D). A few tumors,

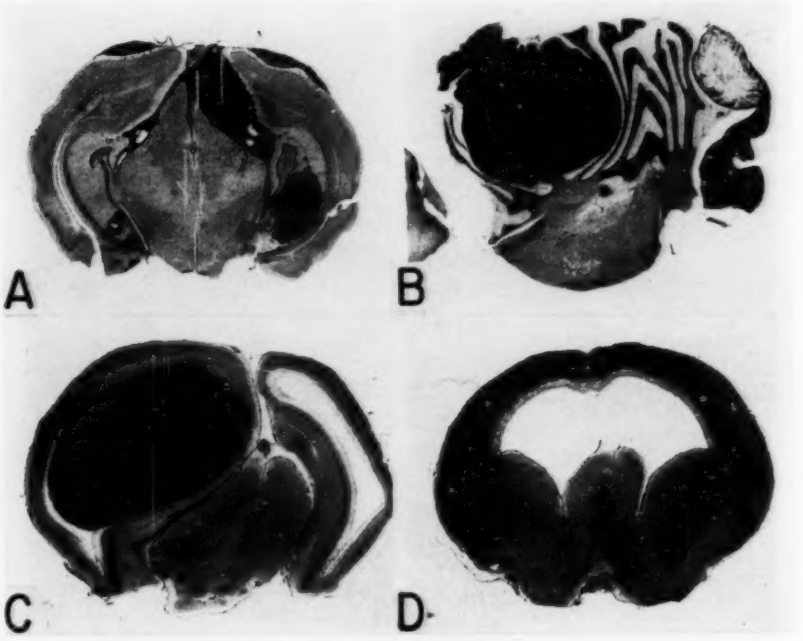

FIG. 7. A. Extensive tumor growth in the leptomeninges, perivascular cords of tumor invading brain, and tumor masses in the hippocampus. H & E × 12. B. Two non-invasive tumor masses compressing and deforming cerebellum. Osteoid is present in the upper portion of the tumor on the right. H & E × 12. C. Large tumor in the left lateral ventricle deforming the diencephalon and compressing the third ventricle. H & E × 12. D. Anterior section through same brain as 3A showing marked hydrocephalus. H & E × 12.

especially those in the cerebellopontine angle, distorted the brain by their growth, but showed no invasion of brain (Fig. 7B).

The tumors appeared to originate in leptomeninges, dura mater, or perivascular connective tissue within the brain. The presence of areas of heavy tumor growth in the same brain suggested the possibility of multifocal origin of the tumor. Only in two hamsters, however, was it possible to demonstrate non-connected tumor masses by studying serial sections of the brains.

The globular dark red lesions in liver and lungs were hemorrhages. Most of these were in early stages of organization. Clusters of anaplastic tumor cells were frequently found inside or at the edge of the accumulations of blood (Fig. 6C). In the lungs, tumor cells were sometimes identified infiltrating non-hemorrhagic regions. Such cells were more easily recognized when they invaded a blood vessel (Fig. 6D).

C. Pathological Findings in Hamsters Injected with Tumor Cells Cultivated in Vitro

Tumors developed at the site of injection in all (9/9) hamsters injected both intracerebrally and subcutaneously with tumor cells grown in tissue culture. In the interscapular region, multiple tumor masses formed along the needle track. The tumor in the left cerebral hemisphere was connected by a thin stalk of tumor tissue growing through the bony defect left by the injecting needle, with tumor over the calvarium.

None of these hamsters developed tumors elsewhere in the body.

Microscopically, the subcutaneous and intracerebral tumors in all of these animals had the same appearance. Their morphological characteristics were similar to those described for the virus-induced subcutaneous tumors. The tumors contained abundant mitoses and showed a moderate degree of invasiveness.

DISCUSSION

Several observations made in this study deserve further comment. Intracranial tumors were produced only after intracerebral injection of virus or tumor cells devoid of virus. Absence of intracranial tumors following subcutaneous injection of virus may be due either to a relative paucity of susceptible cells in the intracranial structures, or to the inability of virus to penetrate the blood-brain barrier.

It is of interest to speculate on the mechanism by which tumors arise at the site of injection of polyoma virus. If the subcutaneous tissues contain relatively few cells susceptible to malignant transformation by this virus, a large concentration of virus would be needed for tumor production. Such a high virus concentration is present only at the site of injection. By contrast, if organs such as heart, liver and lungs contain large numbers of susceptible cells, fewer virus particles would be required to induce tumors.

Several lines of evidence suggest that the multiple tumors found in any one hamster after injection of virus, originate independently and do not represent metastases from tumor formed at the site of injection:
1) Serial sections of hamster brains occasionally showed multiple nonconnected tumors formed at the site of injection. 2) Several isolated tumors of equal size and low invasiveness were frequently found in other organs. 3) Injection of tumor cells lacking demonstrable virus resulted in tumor formation only at the site of injection. This was demonstrated in the present and other (6, 7) studies. 4) Experiments reported elsewhere (6) showed that when virus and homologous immune serum were injected in hamsters simultaneously but at different sites, single tumors developed almost exclusively at the site of injection. If tumors arising distant from the site of injection were due to metastases, antibody to virus should not have pre-

vented tumor formation at distant sites. Hamster sera do not contain antibodies which suppress the growth of tumor cells (7, 8).

All tumors arising in hamsters at the site of injection and elsewhere were sarcomas of a limited morphological variety containing variable amounts of collagen and reticulum. When these studies were begun, it was hoped to learn how many cell types could be induced to produce tumors by the virus. Since a wide variety of tumors was not obtained, this study does not exclude the possibility that in hamsters only one kind of cell gives rise to tumors.

SUMMARY

1. Tumors were produced intracranially, as well as in the heart, lung, liver and kidney following intracerebral injection of polyoma virus in newborn hamsters.

2. Hamsters injected subcutaneously with the same dose of virus, developed tumors at the site of injection, in the heart, lung, liver and kidney, but not intracranially.

3. All tumors were sarcomas producing variable amounts of reticulum, collagen fibers and rarely forming osteoid or bone.

4. Most intracranial tumors showed extensive growth in leptomeninges over wide areas of brain, in addition to forming large expanding masses at preferential sites: interhemispheric fissure, tentorial region, or cerebral hemispheres. The brain substance was invaded mostly by growth of tumor cells in perivascular cords. Non-invasive brain tumors usually occurred as large globular masses in the cerebellopontine angle.

5. Hemorrhagic lesions in liver and lungs contained invasive tumor cells.

6. Hamsters injected intracerebrally and subcutaneously with tumor cells cultivated *in vitro* containing no demonstrable virus, developed tumors only at the site of injection.

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THE ROLE OF INFECTION IN THE PATHOGENESIS OF EXPERIMENTAL UROLITHIASIS

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INTRODUCTION

Experimental evidence has recently been reported which has cast some doubt on the usefulness of the method of Vermeulen and his colleagues (2) for experimental urolithiasis. It has been reported that without infection and with treatment by some indigenous drugs, stone formation is prevented (1).

Statements have been made that associated infection is the main factor in the formation of stones when zinc is introduced into the bladder of experimental animals, and that if the infection can be controlled with aseptic precaution and postoperative antibiotic therapy, stone formation can be prevented (1). The antibiotic therapy in this instance was penicillin and streptomycin.

To investigate the validity of this contention, this present work was undertaken.

MATERIALS AND METHODS

Three groups of Long-Evans male rats weighing 150 to 200 gm. were selected. Over 20 animals were included in each group. Diet of rats in all groups consisted of Fox checkers and water ad libitum.

One group consisting of twenty-four animals was used as control. In this group, instruments, sutures, and abdominal wall were sterilized with alcohol only. Operations were performed under ether anesthesia. Pieces of chemically pure magnesium to be introduced (weighing 15 mg.) were sterilized by autoclave. All animals were sacrificed after four weeks. At this time urine culture was obtained aseptically by aspiration from the bladder with a sterile tuberculin syringe and needle. The stones were removed, blotted, and the dry weight and pH of the urine was recorded.

In the second and third groups more strict measures of asepsis were enforced. Before and after each operation, all the instruments were resterilized. The abdominal wall was sterilized with alcohol and zephiran. Suturing materials and magnesium were sterilized in the autoclave.

From the day of operation until the animals were sacrificed, each group received antibiotics. One group received daily 0.1 ml. intramuscularly injection of combined penicillin and streptomycin (from mixture of 0.5 gm. streptomycin and 400,000 units of penicillin dissolved in 5 ml. of re-distilled water). The other group received 0.1 ml. intramuscularly of streptomycin only (from 0.5 gm. streptomycin dissolved in 5 ml. of re-distilled water).

Other Investigations of Urine: After two weeks all animals were removed from their cages and after exposure to ether fumes, droplets of urine from the urethra were collected in sterile tryptose-phosphate broth tubes. The abdominal wall and urethra were cleansed with alcohol and zephiran before specimens were obtained.

RESULTS

Weight of the stones, pH, urine culture are recorded in Tables I, II and III. The average stone size of the controls was 117.7 mg. The average for the penicillin and streptomycin group was 89 mg. For the streptomycin group an average of 71 was obtained. When five animals in the control group with alkaline pH and positive culture at two weeks, and four weeks were discarded, average for the control group was 94.0 mg.

Statistical analysis (3) shows that $P = .25$ for the difference between penicillin and streptomycin and also streptomycin alone. Compared with the controls, the value for penicillin and streptomycin is $P = .75$, for streptomycin and the controls $P = .30$. Even including the five animals with alkaline pH and positive cultures in the controls, the value for penicillin and streptomycin is $P = .15$.

TABLE I

Vesical Stone Size and Urine Culture in Control Animals

No.	Stone Wt. (mg.)	pH	Culture	
			(4 weeks)	(2 weeks)
1	132	6.7	+	+
2	89	7.0	+	+
3	341	7.6	+	+
4	83	6.8	−	−
5	24	6.8	−	−
6	61	6.4	−	−
7	28	6.4	−	−
8	114	6.4	−	−
9	193	6.9	−	+
10	47	6.4	−	−
11	144	7.1	+	+
12	164	6.5	−	+
13	76	6.8	−	−
14	260	6.8	−	−
15	98	6.8	−	−
16	168	6.4	−	−
17	144	6.1	−	−
18	170	6.6	−	−
19	45	6.9	−	−
20	84	6.8	−	−
21	80	6.3	−	−
22	99	7.1	−	+
23	63	6.2	−	−
24	99	7.1	+	+

TABLE II

Vesical Stone Size and Urine Culture in Streptomycin Animals

No.	Stone (mg.)	pH	Culture	
			(4 weeks)	(2 weeks)
1	72	6.0	−	−
2	150	6.1	−	−
3	154	6.0	−	+
4	38	6.1	+	−
5	91	6.4	−	+
6	117	6.1	−	−
7	31	6.1	−	+
8	7	6.9	−	−
9	28	6.7	+	−
10	67	6.2	−	+
11	83	6.1	−	+
12	158	6.3	−	−
13	20	6.1	−	+
14	43	6.3	−	+
15	74	6.1	−	+
16	64	6.4	−	−
17	14	6.5	+	−
18	117	6.8	−	−
19	27	6.7	−	−

pH of Urine

The pH of the rats in the controls varied from 6.1 to 7.6, only five being on the alkaline side. In the penicillin and streptomycin group, the pH varied from 6.3 to 6.9, none being alkaline. In the streptomycin group pH varied from 6.0 to 6.9, none being alkaline.

Urine Culture

Admittedly, the method of urine culture at the two week interval was quite crude. Contamination was possible from many sources: urethra, abdominal wall, air, and others. Spurious results: i.e. positive culture at two weeks, with no alkaline pH or positive culture at four weeks were quite possible. Only in those instances where positive culture was obtained at both intervals was infection presumed to be present. Five animals in the control group fulfilled this requirement. No animals in the penicillin and streptomycin group had positive cultures at four weeks. In the streptomycin group, eight animals had positive cultures at the two week period. However, a different three animals had positive cultures at the end of the four week period. In neither of the periods was an alkaline pH observed. It is believed these sporadic results are a reflection of only contamination.

The results of this experiment reflected the observation of Vermeulen

TABLE III

Vesical Stone Size and Urine Culture in Penicillin and Streptomycin Animals

No.	Stone (mg.)	pH	Culture	
			(4 weeks)	(2 weeks)
1	172	6.4	−	+
2	94	6.4	−	+
3	71	6.2	−	+
4	89	6.7	−	+
5	75	6.4	−	+
6	61	6.4	−	+
7	88	6.6	−	+
8	91	6.7	−	−
9	71	6.7	−	−
10	68	6.4	−	−
11	51	6.4	−	+
12	81	6.6	−	−
13	115	6.4	−	−
14	270	6.9	−	−
15	44	6.8	−	−
16	45	6.3	−	−
17	118	6.4	−	+
18	62	6.4	−	−
19	77	6.9	−	+
20	108	6.7	−	−
21	13	6.5	−	−

and his associates (2) that experimental production of stones was possible, without infection and pH change, with foreign body (magnesium c.p.) in the rat, following their technique. In the control group the incidence of infection was 20 per cent as demonstrated by culture and pH. These five animals were not utilized in evaluating the data.

The question of spontaneous recovery during the four week period has been mentioned (1). The method of urine culture at two weeks, was an attempt to document this. Only the control group showed any correlation between cultures at the mid-period and termination of the experiment.

If the three animals showing slightly positive cultures at term in the streptomycin group are taken as having air infection, discarding these animals would raise the average stone size and render this group even less significantly different statistically.

Since a reasonable quantity of stone formation was possible after introduction of a piece of magnesium in the bladder of the rat, when adequate aseptic precautions were taken and when adequate antibiotic therapy was continued during the period of experiment, this technique of production of experimental lithiasis was considered effective.

SUMMARY

In three groups of male Long-Evans rats, experimental production of urinary lithiasis was produced by introducing a piece of c.p. magnesium weighing 15 mg. into the bladder of the animals. The animals were sacrificed after four weeks.

In the first group of control rats in 19 animals, showing no evidence of infection, the average stone size was 94 mg.

In the second group of rats receiving streptomycin and penicillin, the average stone size was 89 mg.

In the third group of rats receiving streptomycin only, the average stone size was 71 mg. No significant statistical difference between the three groups was noted.

Urine cultures were taken at two weeks and at four weeks to rule out infection.

It is concluded that experimental production of urinary lithiasis is possible by introducing a piece of magnesium in the bladder of the rat without significant infection. Antibiotic therapy tends to remove early bacterial contamination.

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TOXICITY OF BACTERIAL ENDOTOXINS FOR THE CHICKEN EMBRYO: PATHOLOGICAL AND BIOLOGICAL STUDIES[1]

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INTRODUCTION

This report confirms and extends the observations of Smith and Thomas (1) that relatively small doses of bacterial endotoxin are lethal for the chicken embryo. Detailed histologic studies revealed that the most prominent pathological finding in 13 to 15 days old embryos injected intravenously with endotoxin was extensive hemorrhagic encephalopathy, apparently the result of widespread vascular damage. Cerebral lesions developed sequentially and were characterized by vascular congestion, extravasation of blood or plasma into cerebral ventricles, dilatation of the ventricular system, discrete and confluent brain hemorrhages, and necrosis of nervous tissue.

It was also of interest to determine whether the phenomenon of acquired resistance to the action of bacterial endotoxin (2, 3) could be demonstrated in an immunologically immature host. It was noted that prior injection of a sublethal dose of endotoxin resulted in a slight, but significant, increase in the capacity of the chicken embryo to resist subsequent challenge with a lethal dose of endotoxin.

MATERIALS AND METHODS

Endotoxins. Purified bacterial lipopolysaccharides extracted from *Escherichia coli* 08 ("Kroger" strain) and *Salmonella abortus equi* were supplied by Dr. Otto Westphal of the A. Wander Forschungsinstitut, Sackingen, Germany. Weighed amounts of these materials were suspended in physiologic salt solution and diluted to the desired concentration prior to use. Typhoid vaccine was prepared from the TY2 (Felix) strain of *S. typhosa* and contained approximately 10^9 bacteria per ml. killed by heating at 56°C. for one hour.

Injection of Embryos. Chicken embryos 13 to 15 days of age were used in all experiments. Intravenous injection was made in a large allantoic vein after removing a 0.5 × 1.0 cm. portion of egg shell. A drop of mineral oil was placed on the exposed shell membrane and, with the aid of transillumination, 0.05–0.1 ml. of material was in-

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jected through a 27-gauge needle introduced into the vein in the direction of blood flow. The window in the shell was sealed with a mixture of melted paraffin and beeswax. Allantoic inoculations were made by the standard technique described by Beveridge and Burnet (4). All eggs were incubated at 38°C. before and after injection.

Preparation of Brains for Histologic Study. The jelly-like consistency of chicken embryo brains necessitated a special procedure for partial *in situ* fixation which permitted removal of the cranial contents *en bloc*. Embryos were killed by injecting 1 ml. of 10 per cent formalin into an allantoic vein. Small incisions were made in the skull to assure effective penetration of fixative, and the entire head was then placed in 10 per cent formalin for 24 to 48 hours. After this preliminary fixation, the brain was carefully removed from the cranial cavity and immersed in 10 per cent formalin for two weeks prior to celloidin embedding. Sections were cut serially at 20 to 30 microns and stained with Van Gieson's or Kluver's stains.

EXPERIMENTAL

The Lethal Effect. The response of chicken embryos to bacterial endotoxin is determined by the dose of the inoculum, route of administration, and age of the host (1).

Groups of 20 chicken embryos were inoculated intravenously at 14 days of age with graded doses of *E. coli* lipopolysaccharide. Twenty embryos similarly inoculated with normal saline solution served as controls. After further incubation for 3, 6, 10, 20, 48, and 72 hours, eggs were candled to determine the incidence of death.

As shown in Figure 1, death occurred in approximately 50 per cent of embryos inoculated intravenously with 0.1 microgm. of *E. coli* lipopolysaccharide, and in 90 per cent of embryos inoculated with 10 microgm. of the same material. Regardless of the dose of endotoxin, only occasional deaths occurred prior to 3 hours, or later than 20 hours after injection. A small percentage of deaths was related to the inoculation procedure; repeated experiments showed that intravenous injection of saline solution resulted in death of 5 to 10 per cent of embryos.

Despite careful attention to factors such as embryonic age and temperature of incubation, both shown to be important determinants of the lethal activity of endotoxins (1), considerable variation in the response to equivalent doses of the same endotoxin preparation was observed. For example, in three additional experiments, in which 0.01 microgm. of *S. abortus equi* lipopolysaccharide was injected intravenously in 14 day old embryos, the mortality rates were 23, 37, and 70 per cent.

In contrast to the effect of endotoxin injected intravenously, death occurred only occasionally after allantoic inoculation. Whereas 0.1 microgm. of *S. abortus equi* lipopolysaccharide consistently killed more than 50 per cent of embryos inoculated intravenously, 10 microgm. injected in the allantoic sac was well tolerated.

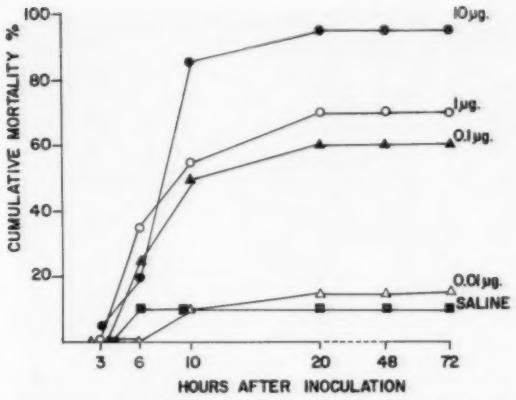

FIG. 1. Lethal effect of graded doses of *E. coli* lipopolysaccharide injected intravenously in 14 day old chicken embryos.

Intravenous injection of typhoid vaccine also caused the death of a large proportion of chicken embryos, whereas equivalent doses administered by the allantoic route had only a negligible effect (Table I). The lethal action of typhoid vaccine appeared to be a consequence of its content of bacterial endotoxin rather than of particulate material. A similar preparation containing 4.4×10^8 heat-killed *Staphylococcus aureus* was innocuous when injected intravenously.

In general, the results of the preceding experiments confirm the findings of Smith and Thomas (1) that minute doses of endotoxin injected by the intravenous route cause death of chicken embryos within 24 hours, and that relatively large doses of endotoxin inoculated allantoically are without lethal effect.

TABLE I

Lethal Effect of Typhoid Vaccine Inoculated Intravenously or Allantoically in 14 Day Old Chicken Embryos

Material	Route	Number of Bacilli Inoculated	Death	
			Number	Per Cent
Typhoid vaccine	IV	7.0×10^8	14/16	87.5
		1.7×10^8	18/27	66.6
		4.4×10^7	15/27	55.5
Saline	IV	—	1/24	4.1
Typhoid vaccine	All	7.0×10^8	4/74	5.4
Saline	All	—	0/57	0

IV = Intravenous.
All = Allantoic.

TABLE II

The Development of Cerebral Hemorrhages in 14 Day Old Chicken Embryos Inoculated Intravenously with 1 μg. E. coli Endotoxin

Time After Injection	Cumulative Mortality		Degree of Gross Brain Hemorrhages*
	Number	Per Cent	
Hours			
0	0/55		
3	1/55	1.8	0
6	10/55	18.1	0,0,0,0,0,0,1,1,2
10	22/55	40.0	1,2,2,2,2,2,3,3,3,3,3,3
20	33/55	60.0	0,0,0,2,2,3,3,3,3,3,3
27	35/55	63.6	0,2
48	37/55	67.2	2,3

* Brain lesions were graded 0, 1+, 2+, or 3+ according to their extent and severity.

Gross and Microscopic Pathology.

Each of 55 chicken embryos was inoculated intravenously at 14 days of age with 1 microgm. of *E. coli* lipopolysaccharide diluted in 0.1 ml. of normal saline solution. Embryos injected intravenously with diluent alone served as controls. At frequent intervals, thereafter the eggs were candled to determine the incidence of death. Each dead embryo was carefully examined for external evidence of cerebral hemorrhages. The gross pathological lesions were scored individually on an arbitrary scale of 0 to 3+, according to their extent and severity (5).

As shown in Table II, 1 microgm. of *E. coli* endotoxin injected by the intravenous route produced death of 67 per cent of embryos within 48 hours. Scattered petechial hemorrhages in skin (1) were commonly encountered, but the most striking gross pathological lesions were intracranial hemorrhages (Fig. 2). The incidence of these brain hemorrhages, and their severity, is also recorded in Table II. As noted, cerebral hemorrhages were uncommon if death occurred within 6 hours. By 10 hours the vast majority of dead embryos exhibited extensive brain hemorrhages. It is of interest that the severity of the lesions did not increase with time beyond 10 hours. Patently, these gross observations alone constitute insufficient evidence for vascular damage as the cause of death. However, studies described below indicate that the lethal action of endotoxin is more closely correlated with microscopic lesions of blood vessels.

Each of 60 embryos was inoculated intravenously at 14 days of age with 1 microgm. of *E. coli* endotoxin. Appropriate controls were injected with saline solution. Four surviving embryos in the group injected with endotoxin and two controls were killed at 3, 6, 10, 20, 27, 48, 72, 96 and 120 hours. The brain of each embryo was carefully examined for evidence of gross and microscopic lesions (see Materials and Methods). Dead embryos were not included in this study.

FIG. 2. Chicken embryo, 15 days old, 24 hours after intravenous injection of 1 μg. of *E. Coli* endotoxin.

Representative examples of the gross appearance of brains of chicken embryos sacrificed at intervals after intravenous injection of 1 microgm. of *E. coli* endotoxin are shown in Figures 3 and 4. No gross pathologic changes were visible three hours after injection (Fig. 3A). However, three of four embryo brains examined 6 hours after injection showed multiple discrete and confluent hemorrhages (Figs. 3B and 3C). Cerebral hemorrhages were also prominent 10 or 20 hours after injection (Fig. 3, D–G) but were not more marked than those in embryos sacrificed after six hours.

The cerebral ventricles of embryos that survived 20 hours or longer were usually greatly distended with blood or plasma. The extraordinary appearance of these late lesions is illustrated in Figure 4, in which the distended lateral horns are clearly visible as symmetrical, crescent-shaped prominences on the posterior-medial aspect of the cerebral hemispheres. Extensive intraventricular hemorrhages were also often present as shown in Figure 3G. In addition to marked ventricular dilatation, brains of embryos sacrificed 48 to 120 hours after injection often showed extensive areas of cystic necrosis at sites of old hemorrhages (Figs. 3H, 3I; and Fig. 4).

The microscopic alterations observed in brains of chicken embryos sacrificed at selected intervals after injection of 1 microgm. of *E. coli* endotoxin are summarized in Table III. The earliest changes were dilatation and

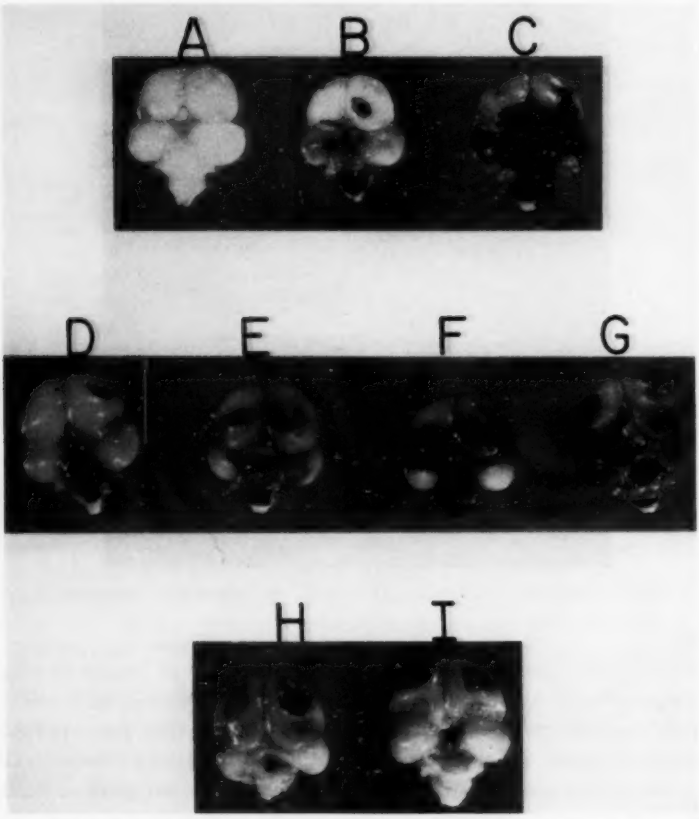

FIG. 3. Brains from chicken embryos 3 (A), 6 (B, C), 10 (D, E), 20 (F, G), 70 (H), and 96 (I) hours after intravenous injection of 1 μg. *E. coli* endotoxin.

congestion of cerebral capillaries and veins. At 3 hours after injection, vascular congestion was prominent especially in the paraventricular areas (Fig. 5) and in the choroid plexus (Fig. 6); 3 of 4 brains also showed extravasation of small amounts of plasma into the ventricles. Multiple petechial hemorrhages (Figs. 7 to 9) and intraventricular extravasations (Figs. 8, 10) were observed regularly 6 or more hours after injection. By that time petechial hemorrhages had become confluent in most brains. Multiple areas of necrotic cerebral tissue (Figs. 8, 9, 11) were prominent in brains of embryos sacrificed 48 to 120 hours after injection.

Brains of embryos injected intravenously with normal saline solution showed only minimal vascular congestion. Hemorrhage and necrosis of tissue was not observed in the controls.

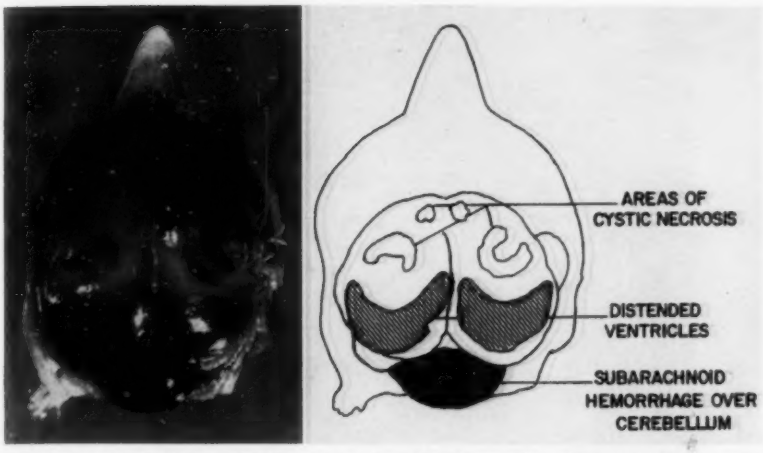

FIG. 4. Head of 18 day old chicken embryo 96 hours after intravenous injection of 1 μg. *E. coli* endotoxin. The skin and superior portion of the skull have been removed to show brain *in situ*.

TABLE III

Microscopic Lesions Observed in Brains of Chicken Embryos Injected Intravenously with 1 μg. of E. coli Endotoxin

Time After Injection	Embryos Examined	Types of Lesion in Each Embryo					
		None	Vascular Congestion	Extravasation of Blood or Plasma into Ventricles	Ventricular Dilatation	Petechial Hemorrhages	Confluent Hemorrhages and Necrosis
hours							
3	4		4	3	3		
6	4		4	4	3	4	2
10	4		4	4	4	4	3
20	4		4	4	4	4	4
27	4		4	4	4	4	3
48	4		4	4	4	4	4
72	4		4	4	4	3	3
96	4		4	4	4	4	4
120	4	1	3	3	3	2	2

Embryos were 14 days old at 0 hours and were living at the time the brains were fixed with formalin.

Acquired Resistance. Many investigators have shown that prior injection of sublethal doses of bacterial endotoxin evokes rapid changes in resistance of the mature host to the lethal action of homologous or heterologous endotoxins (2, 3). The following experiments suggest that acquired

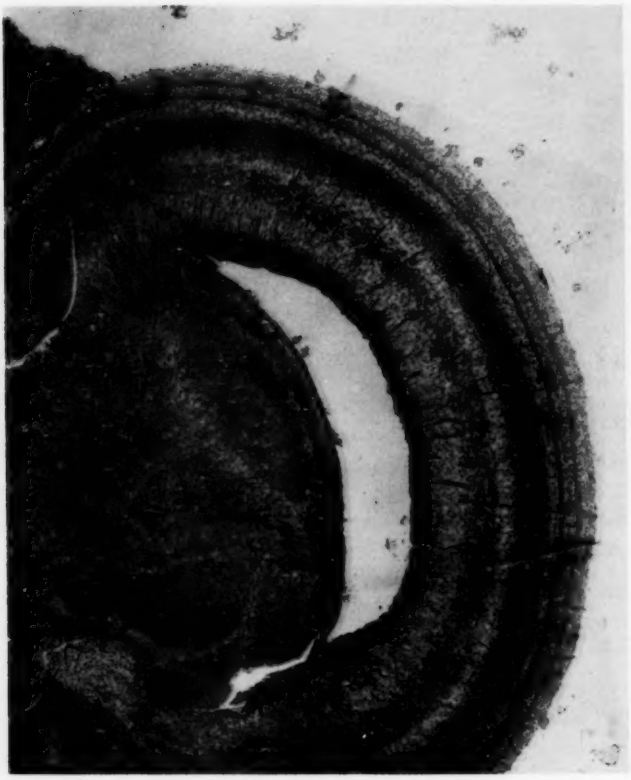

FIG. 5. Section through optic lobe 3 hours after injection of endotoxin showing paraventricular capillary and venous congestion (× 35).

resistance to the lethal action of endotoxin can also be demonstrated in the chicken embryo.

Fourteen day old chicken embryos in groups of approximately 60 were injected by the intravenous route with normal saline solution or a sublethal dose of bacterial endotoxin. After 17 to 24 hours, the eggs were candled and dead embryos were discarded. If 10 per cent or more of the embryos were dead 17 to 24 hours after the initial injection, the experiment was discontinued. Otherwise, embryos pretreated with saline or endotoxin were then challenged with normal saline solution or graded doses of bacterial endotoxin injected into an allantoic vein at a site distant from the initial intravenous inoculation. The doses of endotoxin in the challenge inocula ranged from 5 to 1,000 times greater than the doses of endotoxin in the intial or "protective" inoculum. All experiments were terminated 24 hours after challenge and the final results calculated on the basis of survival for this period of time.

In all, 15 experiments were performed. Five series were not carried to

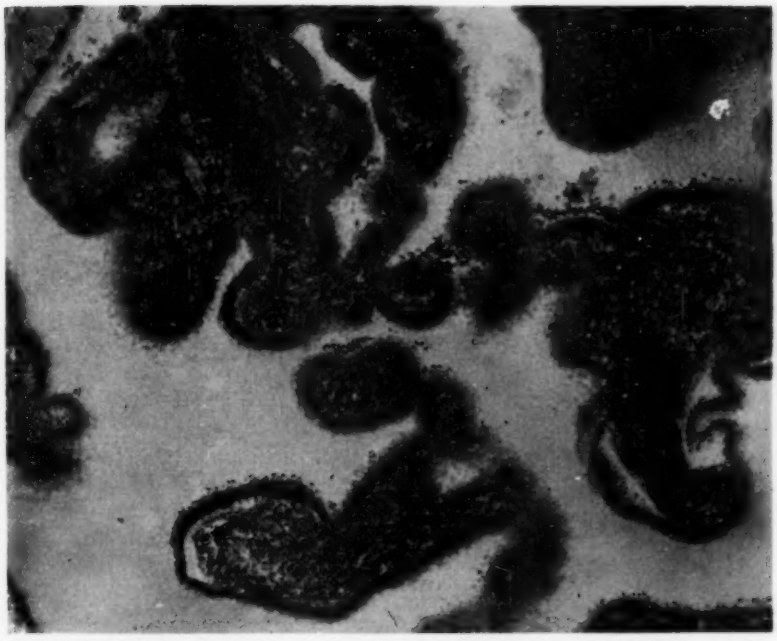

FIG. 6. Marked vascular congestion of choroid plexus 3 hours after injection of endotoxin (× 150).

completion because 10 to 40 per cent of embryos died after the initial dose of endotoxin. It is of interest that in each instance the dose of endotoxin injected had been shown by previous titration not to produce death of embryos of similar age. Three additional experiments were not included because of excessive mortality in the control group of embryos "challenged" with a second injection of saline solution.

The results of the seven adequately controlled experiments are shown in Table IV. The mortality rate of chicken embryos pretreated with sublethal doses of endotoxin 17 to 24 hours prior to challenge with lethal inocula was less than that of controls pretreated with saline solution (Table IV, Experiments 1 to 4). The protective effect of a prior injection of endotoxin was demonstrated repeatedly, but at best consisted of reduction in mortality by one-half that observed in control groups. As shown by experiments 5 to 7 in Table IV, a protective effect was not demonstrated consistently when the time between initial and challenge inoculations was 14 hours or less.

All embryos were examined for the presence of brain lesions 24 hours after challenge, and no significant difference could be detected in the in-

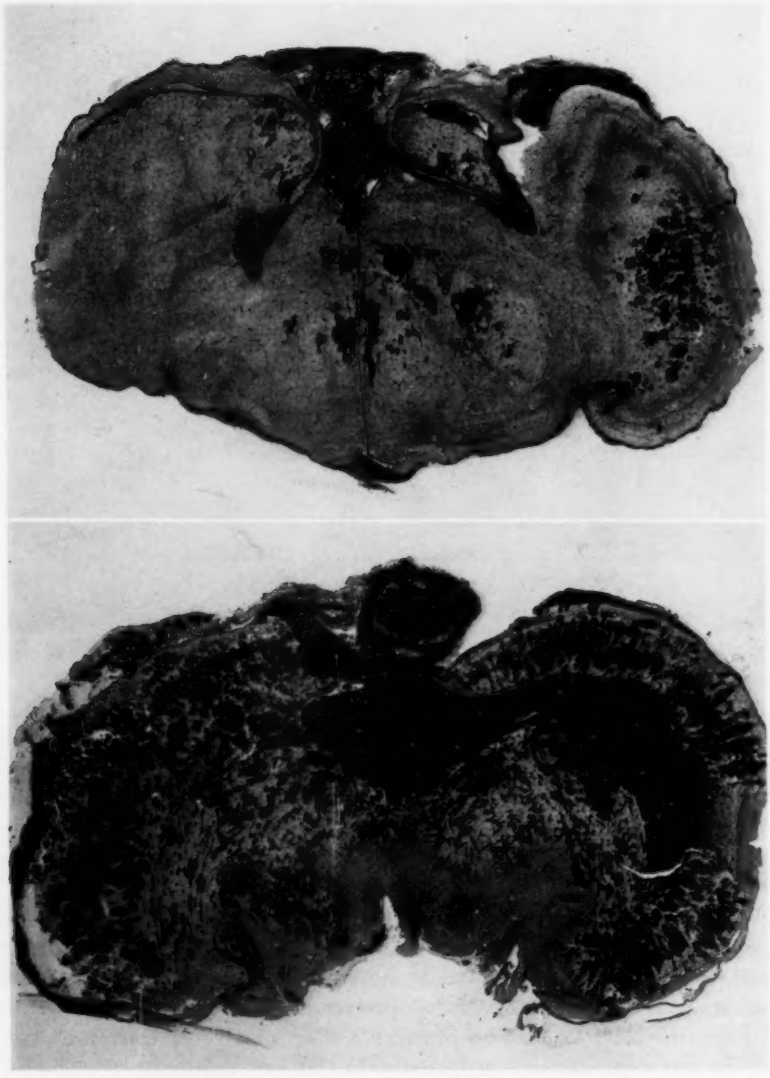

FIG. 7. Section through cerebrum 6 hours after injection of endotoxin showing multiple petechial hemorrhages (× 13).

FIG. 8. Section through cerebrum 20 hours after injection of endotoxin showing coalescent petechial hemorrhages, necrosis and large intraventricular hemorrhage (× 12).

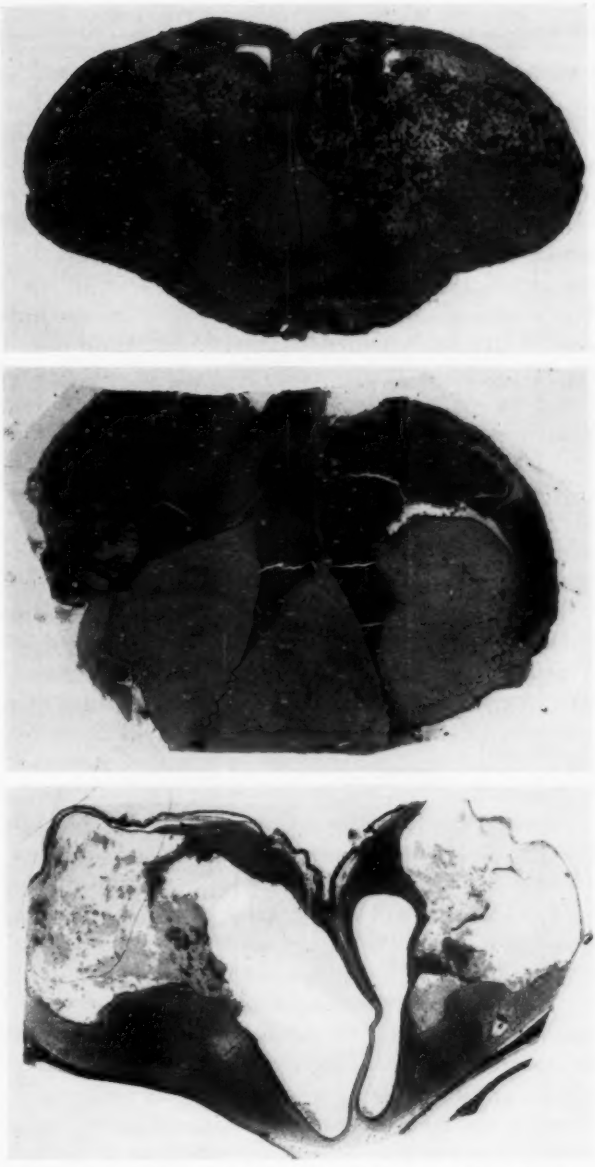

FIG. 9. Section through frontal lobes 20 hours after injection of endotoxin showing coalescent petechial hemorrhages and necrosis (× 13).

FIG. 10. Section through mid-portion of cerebrum 10 hours after injection of endotoxin showing massive intraventricular hemorrhage and dilatation of lateral and third ventricles (× 14).

FIG. 11. Section through frontal lobes 120 hours after injection of endotoxin showing marked dilation of lateral ventricles and bilateral cystic necrosis of frontal lobes (× 12).

TABLE IV

Acquired Resistance to the Lethal Effect of Bacterial Endotoxin in the Chicken Embryo

Experiment No.	Age of Embryo	Interval Between Protective and Challenge Inoculations	Protective Inocula	Challenge Inocula	Mortality After Challenge	
					Number	Per Cent
	days	*hours*				
1	13	24	saline	saline	4/13	30
			"	AE, 0.001 μg.	9/13	69
			"	AE, 0.01 μg.	12/13	92
			AE, 0.0001 μg.	saline	4/13	30
			"	AE, 0.001 μg.	4/14	28
			"	AE, 0.01 μg.	10/14	71
2	14	20	saline	saline	1/16	6
			"	AE, 0.01 μg.	12/17	70
			"	AE, 0.1 μg.	10/14	71
			AE, 0.0001 μg.	saline	1/18	5
			"	AE, 0.01 μg.	6/17	35
			"	AE, 0.1 μg.	10/16	62
3	14	18	saline	saline	1/9	11
			"	AE, 0.01 μg.	3/8	37
			"	AE, 0.1 μg.	8/9	89
			AE, 0.001 μg.	saline	1/12	8
			"	AE, 0.01 μg.	2/9	22
			"	AE, 0.1 μg.	6/11	54
4	14	17	saline	coli, 0.1 μg.	17/20	85
			"	coli, 0.5 μg.	19/20	95
			"	coli, 1.0 μg.	16/16	100
			coli, 0.01 μg.	coli, 0.1 μg.	10/20	50
			"	coli, 0.5 μg.	17/20	85
			"	coli, 1.0 μg.	18/20	90
5	14	14	saline	saline	1/18	5
			"	AE, 0.01 μg.	4/17	23
			"	AE, 0.1 μg.	17/18	94
			AE, 0.0001 μg.	saline	1/16	6
			"	AE, 0.01 μg.	6/16	37
			"	AE, 0.1 μg.	12/16	74
6	15	8	saline	saline	3/17	17
			"	AE, 0.01 μg.	4/17	23
			"	AE, 0.1 μg.	12/18	66
			AE, 0.001 μg.	saline	3/19	15
			"	AE, 0.01 μg.	2/20	10
			"	AE, 0.1 μg.	7/20	35
7	15	3	saline	saline	3/23	13
			"	AE, 0.01 μg.	5/21	23
			"	AE, 0.1 μg.	10/21	47
			AE, 0.001 μg.	saline	5/24	20
			"	AE, 0.01 μg.	2/21	9
			"	AE, 0.1 μg.	17/23	73

cidence of hemorrhagic lesions in those embryos pretreated with endotoxin or saline solution.

The experiments comparing the protective action of saline with sublethal doses of endotoxin, shown in Table IV, were subjected to statistical analysis by considering the data as a series of 2×2 tables, according to the method recommended by Cochran (6). For experiments 1 to 4, in which the interval to challenge was 17 to 24 hours, the combined data gave a P value of <0.0001 for enhanced survival of the groups pretreated with endotoxin. In contrast, analysis of the composite data obtained in experiments 5 to 7, in which the interval to challenge was 3 to 14 hours, revealed a P value of >0.05 in favor of endotoxin over saline. Therefore, these findings indicate that significant, albeit slight, protection is afforded chicken embryos pretreated with sublethal doses of endotoxin, provided that sufficient time elapses prior to endotoxin challenge.

DISCUSSION

Vascular reactions are among the most prominent of the many biological effects produced by endotoxins of Gram-negative bacteria. A single dose of endotoxin injected in a susceptible host by the parenteral route may be followed by vaso-constriction and vaso-dilatation, altered reactivity of terminal blood vessels to epinephrine, shock, hemorrhages in skin and viscera, or hemorrhagic necrosis of malignant tumors (2, 3, 7 to 9). The local and generalized Shwartzman reactions represent even more complex vascular phenomena elicited by bacterial endotoxins (2, 3, 9, 10).

The pathological effects observed after injection of endotoxins in 13 to 15 day old chicken embryos are apparently the consequence of widespread vascular damage. The earliest lesion noted was intense congestion of small blood vessels, followed by capillary rupture and the appearance of discrete and confluent petechial intracerebral hemorrhages and intraventricular bleeding. Necrosis of nervous tissue was often observed in brains of embryos surviving 24 or more hours after injection of endotoxin and appeared to be secondary to the extensive vascular lesions.

Mechanisms underlying the vascular effect of endotoxin on the chicken embryo are obscure. Although it is clear that injection of endotoxin is followed by widespread vascular damage, it is not known whether the effect on blood vessels is direct or whether it involves a series of secondary events. It is of interest that regardless of the dose, there is a lag period of 1 to 3 hours before the development of hemorrhagic lesions and death. This observation is consistent with the possibility that an indirect mechanism may be involved in the production of vascular damage.

Studies of the pathogenesis of the hemorrhagic and necrotizing lesions of the local and generalized Shwartzman phenomena suggest that endo-

toxins are not directly destructive to blood vessels, but produce these reactions by occlusion of small blood vessels with platelet-leukocyte thrombi or precipitated fibrinogen (10 to 12). However, the histopathological data described in the present report provide no evidence that vascular occlusion of this or any other type contributes to the development of hemorrhages in chicken embryos after injection of endotoxins. Hypersensitivity to products of Gram-negative bacteria has been offered as an explanation for certain of the physiological disturbances produced by endotoxins (13, 14). As pointed out by Smith and Thomas (1), it is unlikely that the chicken embryo is hypersensitive to endotoxins and it is more plausible to consider other mechanisms of vascular damage. For example, endotoxin has been shown to alter the reactivity of small blood vessels to epinephrine, and epinephrine produces hemorrhagic necrosis in the skin of rabbits treated with endotoxin (8, 9). Smith and Thomas (1) suggested that the increased susceptibility of 10 day old chicken embryos to the lethal effect of endotoxin might be related to the development of epinephrine-producing tissues at this time.

It is of interest that the phenomenon of acquired resistance (2, 3) to the lethal action of endotoxin can be demonstrated in an immature host. Although the effect was not striking, injection of a sublethal dose of endotoxin rapidly increased the resistance of the chicken embryo to subsequent challenge with a dose lethal for control embryos. Unfortunately, the present studies do not aid in defining the mechanism of acquired resistance to the action of endotoxin. A number of events which could alter resistance to intoxication or infection occur after injection of endotoxin, and it is difficult to determine which plays the dominant role in the protective effect (2, 3, 15).

SUMMARY

Chicken embryos 13 to 15 days old develop extensive brain hemorrhages, hydrocephalus and necrosis of nervous tissue after intravenous injection of minute doses of bacterial endotoxins. These lesions are apparently the consequence of widespread vascular damage produced by endotoxins.

Injection of sublethal doses of endotoxin increases the resistance of chicken embryos to subsequent challenge with a lethal dose of endotoxin.

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RENAL GLUCOSE METABOLISM DURING DIURESIS INDUCED BY INFUSIONS OF HYPOTONIC SALINE[1]

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INTRODUCTION

This paper presents data on net renal glucose metabolism in dogs undergoing diuresis, which was induced by the rapid intravenous infusion of hypotonic saline. The evidence indicates that when not in diuresis, the kidneys generally deliver glucose into the circulating blood, but that during diuresis, the kidneys generally extract glucose from the blood, under the conditions studied. These observations are reviewed in the light of others sources of information, which indicate that the magnitudes of both renal glucose production and utilization may be considerable.

MATERIALS AND METHODS

(a) *Basic Experimental Plan*

Renal arteriovenous glucose differences were studied in nine intact mongrel dogs, both unanesthetized and anesthetized, before, during, and after diuresis induced by the rapid intravenous infusion of hypotonic saline (0.6% NaCl in pyrogen free distilled water)[3]. Catheters were first implanted into the renal vein and aorta or carotid artery. After a period which allowed for recovery from the acute operative procedures, renal arteriovenous glucose differences were measured by sampling renal vein and arterial whole blood simultaneously at 15 to 20 minute intervals. Urine collections were made at 15 to 30 minute intervals, depending on the rate of urine flow. Three or four pairs of blood samples, and two urine samples, were collected before starting the infusions. Sampling was then continued for two and one-half to three hours after the start of the infusion. Further experimental details are given in subsequent sections.

Three groups of dogs have been compared. Group I consists of three female dogs, who were studied in the unanesthetized state two or more days after the initial operative procedure (see below). Group II consists of four dogs, three male and one female, studied under light sodium pentobarbital anesthesia (induced by 30 mg. of the anes-

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[3] 0.6 per cent NaCl was chosen for these studies because its infusion presents the kidneys with a greater stimulus for diuresis than would 0.85 per cent (isotonic) NaCl, whereas 0.6 per cent NaCl does not present any appreciable risk of hemolysis on rapid intravenous infusion.

thetic per kilogram given intravenously and repeated in 30 mg. single doses if the dog began to move). Experiments in these dogs began 45 to 60 minutes after completion of all operative procedures. Group III consists of two dogs, male and female, studied under anesthesia as were dogs in Group II, but which failed to develop a good diuresis in response to the infusion, for uncertain reasons.

In all studies in anesthetized dogs, a tracheal cannula was in place to insure adequate ventilation, and the femoral arterial blood pressure and pulse pressure were continuously monitored by means of a Statham pressure transducer and a Grass Model #5 polygraph recorder.

All dogs were kept on a high protein diet for several days before study, except as noted. All were fasted for 18 hours before study, but had water *ad lib*. All animals appeared well except for dog A, Group I, who is believed to have been developing distemper, characterized by cough and rhinitis, at the time of study.

Hematocrit determinations were made during the experiments. All were in the normal ranges for our dogs (35 to 50%). The hematocrits tended to drop slightly during the infusions, and then to return towards their initial levels.

All dogs were examined immediately post mortem after a large intravenous dose of sodium pentobarbital. The positions of the catheters were verified. No thromboses of the renal veins were found. Both kidneys of each dog appeared grossly similar and normal, and were very nearly of equal weight. The total kidney mass of all dogs fell within the ranges previously given for normal dogs (1a).

(*b*) *Operative Techniques*

The method for catheterizing the renal vein, and for studying renal arteriovenous differences in unanesthetized dogs, has been previously described in detail (2). In brief, a small polyvinyl catheter (outside diameter .060 inches) was introduced into the left renal vein through the inferior vena cava, which was approached by means of an abdominal incision. The technique involved virtually no manipulation of the renal vein or kidney, particularly inasmuch as the placing of a suture in the renal vein, previously described, was omitted. The catheter was small in relation to the lumen of the vein, and there is no evidence of obstruction of renal vein blood flow (see discussion). The spermatic or ovarian veins were ligated. The abdominal incision was carefully closed with cotton sutures in all dogs.

An indwelling catheter was placed in the urinary bladder in all dogs, before study. This was introduced through the urethra in female dogs, or through a stab-wound and purse-string suture near the fundus of the bladder, in male dogs under anesthesia. The female dogs in Group I were episiotomized several days before the vascular catheter implantation procedure.

(*c*) *Infusion Techniques*

Infusions of 0.6 per cent NaCl in pyrogen free water were introduced through the renal vein catheter in unanesthetized dogs, and into a branch of the femoral vein in anesthetized dogs. Infusions were given at rates varying between 6.2 and 18.2 ml. per minute, and lasted 30 to 75 minutes. All dogs received 23.4 to 50.4 ml. of fluid per kilogram of body weight. Details for each dog are given in Results (Table I).

(*d*) *Collection of Samples and Analytical Techniques*

All whole blood and urine samples were analyzed for glucose. Whole blood analyses were performed by a precise and specific enzymatic method (2). In this method, the

TABLE I

Volumes and Rates of Infusion

Dog	Weight (kg.)	Sex	Group	Total Infusion (ml.)	Time period of Infusion (minutes)	Average Rate of Infusion (ml./min.)	Total ml. per kg.
A	14.6	F	I	340	55	6.2	23.4
B	12.8	F	I	520	75	6.9	40.6
C	20.0	F	I	520	60	8.7	26.0
I	17.0	F	II	500	30	16.6	29.4
II	15.0	M	II	750	45	16.6	50.0
III	11.4	M	II	575	35	16.4	50.4
IV	16.3	M	II	660	41	16.1	40.5
V	19.0	F	III	500	30	16.6	26.3
VI	26.6	M	III	1000	55	18.2	37.5

whole blood was mixed with trichloracetic acid within less than one minute after collection, which stopped glycolysis immediately. As noted, the renal vein and arterial samples were drawn simultaneously. Statistical evidence previously presented (2) indicates that a single arteriovenous difference measurement will be within 1.8 mg. per cent of the true value, 95 per cent of the time. All data reported here are based on two separate analyses of the whole blood filtrates, each analysis itself done in duplicate. The results of each analysis were then averaged.

Urine glucose determinations were done by the method of Froesch and Renold (3) which corrects enzymatically for non-glucose reducing substances in urine. All urine samples were collected by emptying the bladder as completely as possible. After recording the total volume, aliquots were placed in a quick freezing compartment a few minutes after collection, and stored frozen until the time of glucose assay.

RESULTS

Table I presents detailed data on each dog included in this study, showing the sex, weight, conditions of the experiment (i.e. Group), and the amount and rate of infusion given. Blood pressure and pulse pressures remained normal and stable throughout the experiments in Groups II and III. They were not measured in dogs of Group I.

Figure 1 shows the data obtained from dogs of Group I (unanesthetized) and Group II (anesthetized), showing renal vein-arterial glucose differences[4] (RV-A) and urine flow rates plotted against time. In order to facilitate the presentation and statistical analysis of the data, it was found convenient to pool the raw data into time periods, as follows: "Zero time" for each experiment is taken as the time at which the infusion was started.

[4] Throughout this report, the arteriovenous differences are presented as the difference between the renal vein glucose concentration minus the arterial, or the renal vein-arterial glucose difference (RV-A), all expressed as mg. glucose per 100 ml. of whole blood. A positive sign thus indicates that the renal vein glucose concentration is higher than the arterial, and a negative sign indicates that the renal vein glucose concentration is lower than the arterial.

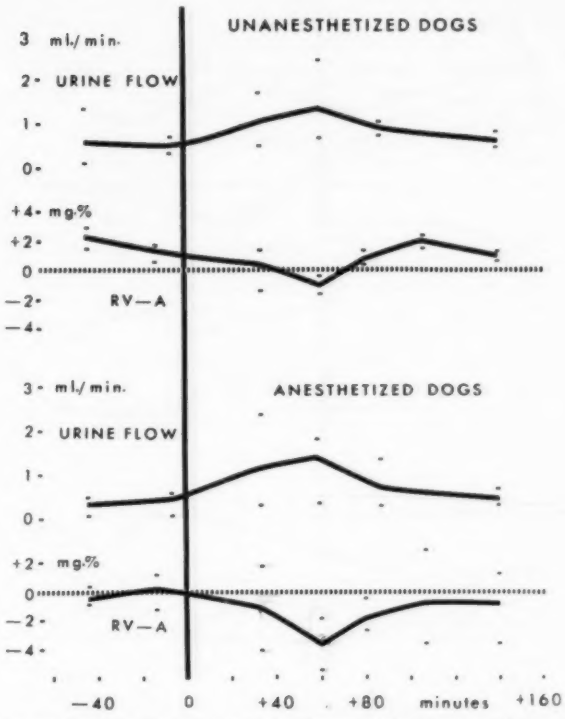

FIG. 1. Mean urine flow rates and renal vein-arterial glucose differences (RV-A) for dogs of Groups (I) (unanesthetized) and II (anesthetized). The vertical line at "0" indicates the time at which infusions were started. See text for other details.

The arteriovenous differences were then pooled into the following time periods; −59 to −30 minutes, −29 to 0, +20 to +49, +50 to +70, +71 to +90, +91 to +120, and +121 to +180 minutes. The urine flow rates, as recorded in the middle of each collection period were pooled into comparable periods as follows: −70 to −20 minutes, −19 to +10, +15 to +45, +46 to +70, +71 to +100, and +101 to +160 minutes. In cases where two or more values from a single dog fell into a given time period, these were averaged first, and that average then used in calculating the mean for the period. Each dog is thus weighed only once in each period. The means for each time period are plotted as in the middle of the period. The ranges of values (highest and lowest for each period) are plotted as small points above and below the lines.

It can be seen that in both groups, the mean pre-infusion renal vein-arterial glucose differences hovered near zero (Group II) or above (Group I). Following the beginning of the infusion, the urine flow rates rose,

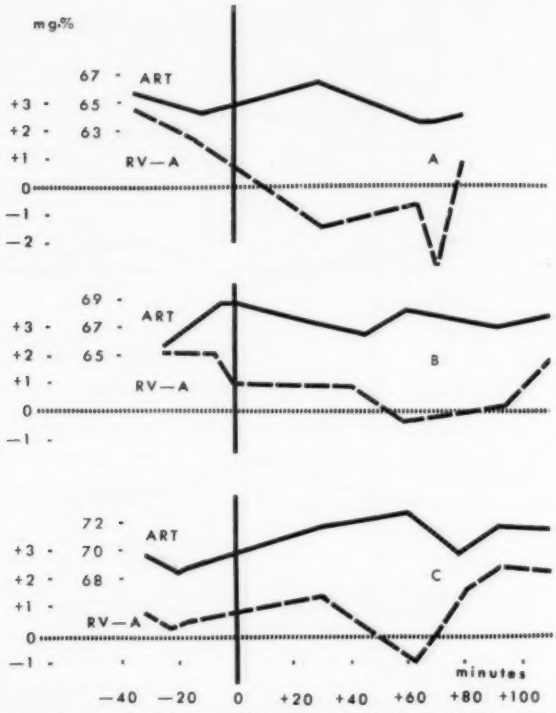

FIG. 2. Renal vein-arterial glucose differences (RV-A, left-hand vertical scales) and the corresponding arterial glucose levels (ART, right-hand vertical scales), all recorded as mg. glucose per 100 ml. of blood, are shown for each dog in Group I, plotted at the time at which the samples were drawn. Infusions started at time "0". Collections were continued past the 100 minutes shown, but all critical changes in RV-A are shown. See text for further discussion.

reaching a peak in the +46 to +70 minute period. Simultaneously the renal vein-arterial glucose differences began to drop reaching a maximally negative value in the +50 to +70 minute period. Following this, the diuresis fell off and the renal vein-arterial glucose differences returned towards control levels.

Figures 2 and 3 show individual plots of the renal vein-arterial glucose differences found in each dog in Groups I and II respectively, compared with the corresponding arterial glucose concentrations, at each time of sampling. It is clear that in Group I (Fig. 2) the arterial glucose levels were quite stable. In Group II (Fig. 3) arterial glucose levels were somewhat less stable, but no regular relationship between changes in arterial glucose level and change in renal vein-artery glucose difference is seen.

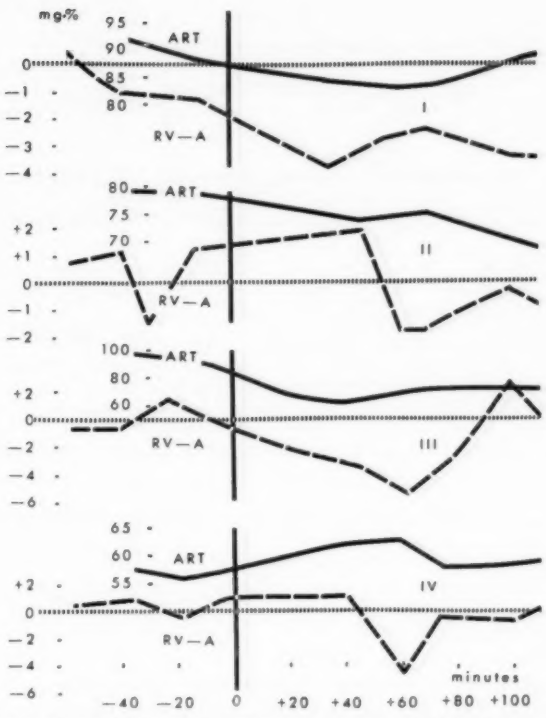

FIG. 3. Renal vein-arterial glucose differences compared with the corresponding arterial levels for all dogs in Group II. See legend for Figure 2 and text for discussion.

Since the total number of animals in each of Groups I and II was small, and because the patterns of response in both groups were quite similar, it seemed justifiable for statistical purposes to pool the data from both groups. The data from each of the comparable periods obtained from all seven dogs in Groups I and II are summarized in Table II, which shows the means and standard errors in each period. Using Student's "t" test (4), the results obtained in each of three periods were compared, and this comparison is shown in Table III. The mean peak urine flow rate (period #4) differed significantly from that in the second pre-infusion period (period #2), and also from that in the last collection period (period #6), at which time urine flow rates had reverted essentially to pre-infusion levels. Similarly significant changes in the mean renal vein-arterial glucose differences were seen at comparable time periods. No significant differences were seen in the means of either set of measurements in the first or last two collection periods (cf. Table II).

TABLE II

Pooled Data From Dogs in Groups I and II

	Period						
	1	2	3	4	5	6	7
Renal Vein-Arterial Glucose Differences (Mg. Per Cent)							
Interval (minutes)	−59, −30	−29, 0	+20, +49	+50, +70	+71, +90	+91, +120	+121, +180
Mean	+.9	+.6	−.5	−2.5	−.6	+.2	−.2
Standard Error	.51	.39	.84	.72	.60	.98	.80
n	7	7	7	7	7	6	6
Urine Flow Rate (Ml. Per Minute)							
	Period						
	1	2	3	4	5	6	
Interval (minutes)	−79, −20	−19, +10	+15, +45	+46, +70	+71, +100	+101, +160	
Mean	.5	.4	1.1	1.3	.7	.5	
Standard Error	.16	.08	.33	.27	.19	.09	
n	7	7	6	7	5	6	

TABLE III

Statistical Comparison of Selected Periods Shown in Table II Giving Student's "t", Degrees of Freedom (d.f.), and Probability that the Differences in the Means Occurred by Chance

Periods Compared	t(d.f.)			
	RV-A	Urine Flow	Probability	
			RV-A	U_f
2 vs 4	3.78 (12)	3.23 (12)	$<.005$	$<.01$
4 vs 6	2.30 (11)	2.64 (11)	$<.05$	$<.025$
2 vs 6	0.39 (11)	0.84 (11)	$>.5$	$>.4$

That the change in renal vein-arterial glucose difference is truly associated with the onset of diuresis is indicated by the results seen in the two dogs of Group III, which did not develop a significant diuresis. The renal vein-arterial glucose differences and urine flow rates for these dogs are plotted against time in Figure 4. It can be seen that little diuresis occurred, and that the renal vein-arterial differences remained generally positive.

Quantitative urine glucose measurements performed on aliquots of all urine samples collected showed that in no dog did a urine glucose concentration higher than 60 mg. per cent occur at any time, and that during diuresis the urine glucose concentrations regularly fell to levels no higher

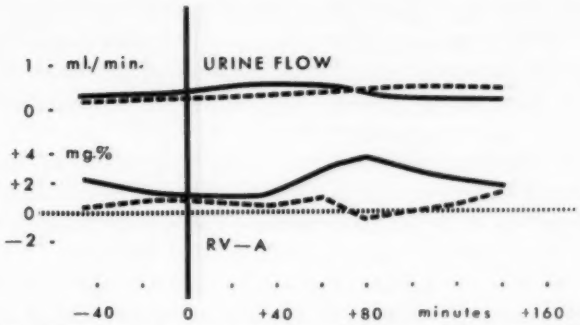

FIG. 4. Urine flow rates and renal vein-arterial glucose differences (RV-A) for dogs V (solid lines) and VI (broken lines), Group III. See text for discussion.

than 30 mg. per cent. It thus appears that in these experiments changes in urinary glucose losses cannot explain the changes in renal vein-arterial glucose differences during diuresis. This and other factors which might influence the results are discussed more extensively below.

DISCUSSION

In a previous report, McCann and Jude (2) confirmed earlier reports that the kidneys of unanesthetized dogs, not in diuresis, produce glucose and deliver this "new" glucose into the renal vein. In such dogs the renal vein glucose concentration was roughly 1 mg. per cent higher than the arterial, on the average, which compares well with the pre-infusion observations made in the present study (Table II, period #1). In the earlier study, the arterial glucose levels were fairly stable and the observations were repeatable hour after hour and day after day. Evidence was presented that hemoconcentration of renal vein blood during urine formation could not account for the positive renal vein-arterial differences noted. Evidence was also presented that the operative procedures did not result in abnormal blood flows in the catheterized kidneys. It was estimated that the net renal glucose production was roughly between 4 and 13 per cent of the hepatic glucose output.

As shown in the present study, rapid intravenous infusions of hypotonic saline (0.6%) caused a change in renal vein-arterial glucose differences from generally positive to uniformly negative values, with the most negative values occurring near the peak of diuresis. This was followed by a return of the arteriovenous glucose differences towards control levels as the diuresis fell off. These findings indicate that a change in net renal glucose metabolism occurred in association with the diuresis, but the point requires some further clarification, nonetheless.

During diuresis, with stable arterial glucose levels, four events might

occur which could alter renal arteriovenous glucose differences. These are 1) a change in renal blood flow, 2) a change in urinary losses of glucose, 3) a change in the hemoconcentration of renal blood due to increasing rates of urine formation, and 4) a change in net renal glucose metabolism. These possibilities are assessed separately below.

(1.) An increase in renal blood flow without a concurrent change in net renal glucose metabolism would decrease the arteriovenous glucose differences regardless of the initial difference. Increasing the renal blood flow towards infinity would bring the arteriovenous differences towards zero, but could never, in itself, change positive values to negative values, or the reverse. Decreasing renal blood flow would, of course, exaggerate the initial difference, but again could not cause a change in the sign of the difference. It has been established by others that the normal diuretic response to water loads is not attended by great changes in either glomerular filtration rate or renal blood flow (1b). Although these were not measured in this study, it seems unlikely that such changes of any magnitude occurred, particularly in view of the fact that all of the anesthetized dogs retained stable, normal blood pressures and pulse pressures throughout the experiments. Furthermore, the technique of catheter implantation requires very little manipulation of the renal vein, and a postoperative equilibration period is routinely allowed. In addition, the catheter, of small diameter in relation to the renal vein, occupied only the last 3 to 4 millimeters of the length of the vein, and no signs of distention, pallor, or cyanosis were seen in the catheterized kidneys. For all these reasons one may assume that the renal blood flows were normal at all times.

(2.) An increased loss of glucose into the urine during diuresis could explain the changes in the arteriovenous differences. However, the results of urine glucose determinations, described previously, seem to preclude this possibility in this case. An example may emphasize this point. The highest single urine glucose concentration seen in any dog during diuresis was 30 mg. per cent. The highest urine flow rate was 2.4 ml. per minute. If a 10 kg. dog, with an estimated total renal blood flow of 250 ml. per minute (2), excreted each minute a total of 2.5 cc. of urine containing 30 mg. per cent glucose, the urinary glucose loss in itself would account for a renal vein-arterial glucose difference of $-.3$ mg. per cent. This is one-eighth of the average difference seen in the $+50$ to $+70$ minute period (pooling data as shown in Table II). It thus seems clear that urinary glucose losses cannot explain our results.

(3.) Changes in renal hemoconcentration also fail to explain our findings. Increased urine flow, in the absence of a comparable change in renal blood flow, would cause an increased renal hemoconcentration, and this would tend to make the renal vein-arterial glucose differences more posi-

tive. Our results, of course, show the reverse. The renal vein-arterial glucose differences moved from positive to negative values during diuresis.

(4.) The fourth possibility, that a change in net renal glucose metabolism occurred in association with the diuresis induced by the infusions, seems to be the only plausible explanation of our results.

It is thus apparent that the kidney can produce glucose when not in diuresis, but that during diuresis induced by infusions of hypotonic saline there is a change in renal metabolism from net glucose production to net glucose uptake. Although the renal arteriovenous glucose differences are small, consideration of the rate of renal blood flow and the mass of renal tissue indicates that the magnitudes of both renal glucose production and uptake can be large. It is of interest to compare estimates of the magnitude of these phenomena with representative figures for the glucose output of liver, the major source of blood glucose, and for the glucose uptake of brain, an avid glucose user. In the subsequent calculations, the following figures have been used: Total renal blood flow in dogs, 25 ml. per kilogram of body weight per minute (2); total renal mass in dogs, 7 grams of kidney per kilogram of body weight (1a); fasting hepatic glucose output in dogs (and man), 150 mg. per kilogram of body weight per hour (2, 5, 6) which is 2.5 mg. per kilogram of body weight per minute or .085 mg. per gram of liver per minute[5]; brain glucose uptake, 5 mg. per 100 grams of brain per minute (in man) (8); renal venous glucose concentration minus the arterial, +.9 mg. per cent in the absence of diuresis (Table II, period 1) and —2.5 mg. per cent at the peak of diuresis (Table II, period 4). Using these figures, the net renal glucose production in dogs not undergoing diuresis is 0.225 mg. per kilogram of body weight per minute, or 0.032 mg. per gram of kidney per minute. This net renal glucose production is thus 9.0 per cent of the total fasting hepatic output, or 37.6 per cent of the hepatic rate on a unit weight basis (mg. of glucose per gram of tissue per minute). The net renal glucose uptake at the peak of diuresis, using the above figures, is 0.625 mg. per kilogram of body weight per minute, or 0.089 mg. per gram of kidney per minute. This is equal to 25 per cent of the total fasting hepatic glucose output, and exceeds the rate of glucose uptake by the brain on a unit weight basis (mg. glucose per gram of tissue per minute).

Paradoxically, the peak renal glucose utilization calculated above is still a small fraction of the glucose delivered by the renal arterial blood each minute. Assuming an arterial blood glucose concentration of 80 mg. per

[5] This figure is based on the following: Liver mass in dogs has been given as 2.94 gm. per 100 gm. body weight, or 29.4 gm. per kilogram (7). A fasting hepatic glucose output of 150 mg. per kilogram body weight per hour would thus represent an output of $150/29.4 \times 60$ mg. or .085 mg. per gram of liver per minute.

cent or 0.8 mg. per ml., and taking the figure for renal blood flow given previously, one can estimate an arterial glucose supply to the kidneys of 20 mg. per kilogram body weight per minute. A peak renal glucose utilization of 0.625 mg. per kilogram body weight per minute would thus be 3.1 per cent of that supplied. In other words, 96.9 per cent of the glucose delivered to the kidneys in the arterial blood should appear in the renal veins. This figure is close to that of 97.8 per cent given by Chinard, *et al.* (9), who measured the recovery of C^{14} labelled glucose in the renal vein after rapid infusions of this material into the renal artery, in dogs.

Nonetheless, sight must not be lost of the fact that the total renal glucose requirements may be very large, under some circumstances, as indicated previously. In addition, no attention has yet been given to the possibility that glucose production and utilization can occur in the kidney simultaneously, perhaps in different anatomical areas. If such occurs, the glucose uptake of some parts of the kidney must be greater on a unit weight basis than is indicated above, and the glucose production must also be greater.

On purely teleological grounds, it is tempting to speculate that the phenomenon of renal glucose production is in some way related to the metabolic support of diuresis, perhaps by ensuring adequate glucose supplies to structures within the kidney which may have large glucose requirements. As suggested above, it is conceivable that an anatomical separation of the sites of glucose production and utilization exists. In addition, the anatomy of the intrarenal circulation (10) appears to be such that glucose formed in one part of the nephron might be delivered to other parts. Unfortunately, however, we do not possess enough information about possible anatomical sites of renal glucose production and uptake, or on the details of the intrarenal circulation to make further speculation profitable at this time. These matters will have to be explored in future studies. The best that can be said at present is that the rates of glucose production and uptake within the kidney as a whole appear to be of considerable potential and that under ordinary circumstances a fairly good balance between the two appears to be struck. The preceding thoughts are presented primarily to indicate a line of investigation which appears to be worthy of pursuit.

SUMMARY

(1) As a step in obtaining further information on the role of glucose metabolism in renal function, serial measurements of renal arteriovenous glucose differences have been made in intact dogs, before, during and after diuresis induced by rapid intravenous infusions of hypotonic saline.

(2) In both unanesthetized and anesthetized dogs, the data indicate that in the absence of diuresis the kidneys usually release more glucose into the renal vein than is delivered by the renal arteries, but that during diu-

resis (after infusions of hypotonic saline) the kidneys regularly extract glucose from the blood.

(3) It is concluded that a change in net renal glucose metabolism occurs during this diuresis, and evidence in support of this conclusion is given.

(4) The data are reviewed in the light of other sources of information which indicate that the rates of renal glucose production and utilization may be of considerable magnitude.

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THE ANTIMICROBIAL SENSITIVITY OF GRAM-NEGATIVE PATHOGENS[1]

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The advent of antibiotics has shifted the spectrum of serious bacterial infections from those caused by the pneumococcus and streptococcus to the staphylococcus and Gram-negative enteric bacteria. While the relative frequency of serious infections with these two groups of organisms is difficult to assess, Gram-negative pathogens have received relatively scant attention while the staphylococcus has occupied the limelight. Several recent reports, however, have focused attention on the increasing prevalence and seriousness of infections caused by Gram-negative bacilli. Finland (1) has emphasized that during the past several years Gram-negative bacteria were responsible for the majority of lethal infections at the Boston City Hospital and Rogers (2) has described a similar trend at The New York Hospital.

Our increasing preoccupation with these bacteria in the clinic and on the wards has led to this review of the antibiotic sensitivity patterns of 621 Gram-negative organisms isolated from patients with clinical infections during the past three years. The majority of organisms were obtained in a nosocomial milieu and, in general, are more resistant than those described by others (3). No attempt will be made in this communication to correlate patterns of antimicrobial sensitivity with results of therapy. It is hoped, however, that a survey of this type will result in a more accurate choice of antibiotics in patients with infections caused by these bacteria.

MATERIALS AND METHODS

Organisms. 621 strains of Gram-negative bacilli isolated from patients with clinical infections between January 1, 1957 and January 1, 1960 were studied. The species of bacteria and their source are recorded in Table I. The majority of strains came from patients with urinary tract infections. This does not reflect the relative frequency of these infections compared to those in other foci since we were particularly interested in urinary tract sepsis and studied more patients with this type of infection. Organisms were identified by routine bacteriologic techniques before sensitivity tests were performed.

Antibiotic Sensitivities. All organisms were tested against penicillin, tetracycline, chloramphenicol, streptomycin, polymyxin B, and either neomycin or kanamycin. A few strains were tested against both of the last-mentioned drugs and some assays were

[1] Supported by a contract with the Army Chemical Corps, Fort Detrick, Md. and in part by Training Grant E-5 of U.S.P.H.S.

TABLE I

Sources of Gram-Negative Pathogens

	Number of Strains						
	Blood	Urine	Resp. Tract	Deep Abscesses	Body Fluids	Other	Total
E. coli	4	173	7	2	2	1	189
Klebsiella-aerogenes	5	76	8	3	2	1	95
Paracolon species	2	28	6	1	1	0	38
Pseudomonas	7	28	11	7	5	1	59
Proteus mirabilis	4	146	2	9	2	0	163
Proteus (indole +)	1	53	0	3	1	0	58
B. Anitratum	1	2	0	1	1	0	5
Serratia	0	0	2	0	0	0	2
Achromobacter	4	1	0	1	0	0	6
Salmonella	3	0	0	0	2	1	6
Total	31	507	36	27	16	4	621

also performed with novobiocin and colistin. A tube-dilution technique, measuring both bacteriostatic and bactericidal end-points was employed (4).

RESULTS

E. coli. One hundred and eighty-nine strains were tested for susceptibility to the action of penicillin, tetracycline, chloramphenicol, streptomycin, neomycin and polymyxin B (Fig. 1). Of note is the fact that nearly 50 per cent of strains were sensitive to 50 units of penicillin per ml., a level which can easily be attained in the body by the administration of large doses of the drug intravenously coupled with probenecid (5). The majority of strains were inhibited by tetracycline and chloramphenicol but neither drug exerted a significant bactericidal effect. Streptomycin, on the other hand, was bactericidal at the same concentration at which inhibition of bacterial growth took place. Between 20 and 45 per cent of all strains were resistant to these three commonly-used drugs at blood levels which are feasible *in vivo* (6). Both neomycin and polymyxin B were bactericidal for the majority of strains. The former, however, was only effective in very high concentration.

One hundred and fifty strains of *E. coli* were also tested against novobiocin; 3 were inhibited by 50 microgm. per ml. and 9 by 100 microgm. per ml. The remainder were resistant to greater than 100 microgm. per ml. Kanamycin was tested against 24 strains; its spectrum closely approximated that of neomycin. Colistin was an effective bacteriostatic agent and 9 of 11 strains were inhibited by 5 microgm. per ml.

Klebsiella (*Aerobacter Aerogenes*). Ninety-five strains of the Klebsiella-

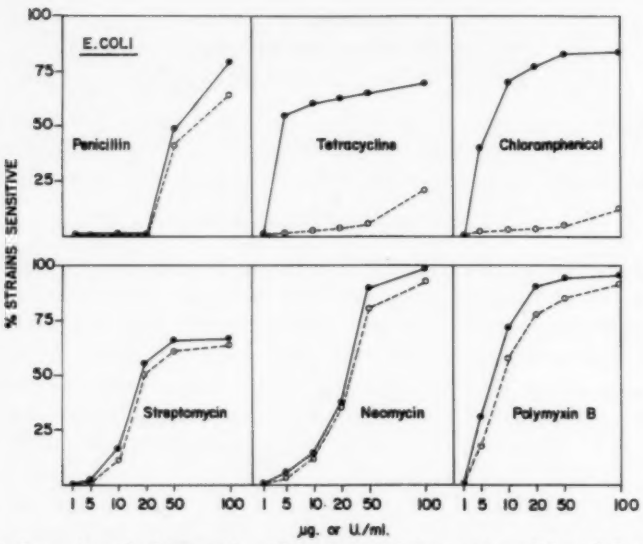

FIG. 1. Bacteriostatic (●—●) and bactericidal (○—○) activity of 6 antibiotics against *E. coli*.

Aerobacter group were tested (Fig. 2). Penicillin was almost entirely ineffective and even in a concentration of 100 microgm. per ml. inhibited less than one-fourth of the strains. Although tetracycline and chloramphenicol possessed relatively potent bacteriostatic activity, they were bactericidal in only a few instances. Streptomycin was active against approximately one-third of strains at feasible tissue levels. Neomycin and polymyxin B showed excellent bacteriostatic and bactericidal activity against this species. At a concentration of 10 microgm. per ml. the former inhibited growth of 63 per cent of strains and the latter 50 per cent.

Although only 26 strains were tested against kanamycin, 23 of 26 were inhibited by 10 microgm. per ml. and 18 of these 23 were killed. Colistin was equally effective and 12 of 15 strains tested were inhibited by 5 microgm. per ml.; for 10 of the 12 the drug was bactericidal. Fifty-six strains were tested against novobiocin. In 47 the organisms grew in a concentration of 100 microgm. per ml. and no strain was inhibited by less than 20 microgm. per ml.

Paracolon Species. Only 38 strains were tested. In general, they tended to be more resistant than *E. coli* and Klebsiella. Penicillin and streptomycin were quite ineffective against these organisms and the other four agents did not inhibit growth of a majorfity of strains unless very high concentrations were reached (Fig. 3). Neomycin appeared to be the most

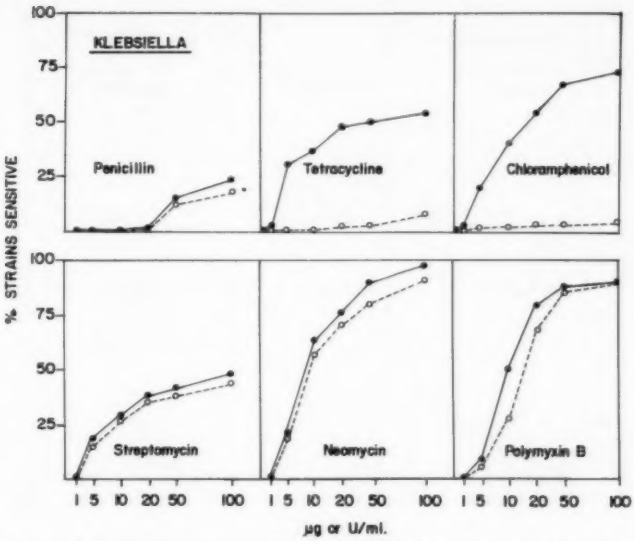

FIG. 2. Bacteriostatic (●—●) and bactericidal (○—○) activity of 6 antibiotics against Klebsiella.

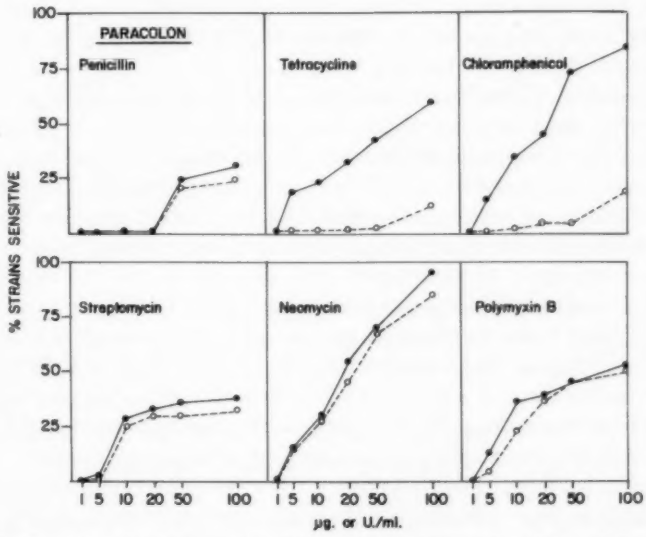

FIG. 3. Bacteriostatic (●—●) and bactericidal (○—○) activity of 6 antibiotics against Paracolon species.

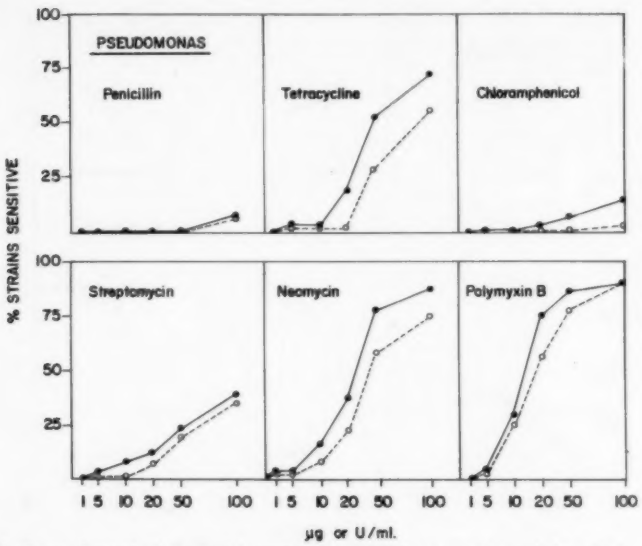

FIG. 4. Bacteriostatic (●—●) and bactericidal (○—○) activity of 6 antibiotics against Pseudomonas.

active of the antimicrobials tested. Although the sensitivity of these strains to kanamycin was not determined, results comparable to those with neomycin might be anticipated because kanamycin and neomycin have quite similar antimicrobial spectra (7).

Pseudomonas. These organisms have a justified reputation of marked resistance to antimicrobial drugs. As shown in Figure 4, penicillin and chloramphenicol in concentrations of 100 microgm. per ml. were almost totally ineffective as inhibitors of growth of Pseudomonas. On the other hand, a number of strains were inhibited by tetracycline which at high concentrations was bactericidal at almost the same concentration at which it was bacteriostatic. Although only an occasional strain was inhibited by low concentrations of streptomycin, approximately 25 per cent of the organisms were killed by 50 microgm. per ml., a level frequently obtained in urine (8). Neomycin and polymyxin B were bacteriostatic for the majority of strains but this effect was achieved only with relatively high concentrations of these drugs. The findings with colistin were of considerable interest. All 10 strains tested were killed by 10 microgm. per ml. of this drug, which is biologically and chemically analogous to polymyxin B (9). Kanamycin's activity was disappointing and no strain was inhibited by less than 50 microgm. per ml.

Proteus mirabilis. One hundred and sixty-three strains, the majority

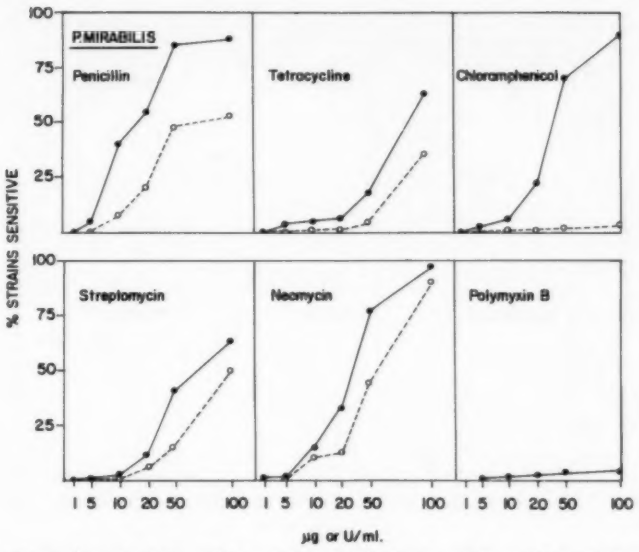

FIG. 5. Bacteriostatic (●—●) and bactericidal (○—○) activity of 6 antibiotics against Proteus mirabilis.

from patients with urinary tract infections, were tested against the antibiotics illustrated in Figure 5. Of considerable interest was the sensitivity of this species of Proteus to penicillin. Forty per cent of the strains were inhibited by 10 units and 85 per cent by 50 units of the drug. Bactericidal activity was nearly as great as bacteriostatic. No other agent approximated penicillin in effectiveness against these organisms particularly when the results were evaluated in terms of clinically attainable blood levels. Although chloramphenicol, streptomycin and neomycin inhibited growth of many strains of *P. mirabilis*, the concentration of drug necessary for bacteriostasis was usually high and was often greater than that achieved with safety *in vivo*.

Fifty strains of *P. mirabilis* were also tested against novobiocin, an agent reported to possess appreciable activity against this species (10). Growth of only 2 of 50 strains was inhibited by 10 microgm. per ml. and 30 of 50 grew in 100 microgm. per ml.

Kanamycin and neomycin showed approximately the same degree of activity. Polymyxin B and colistin were totally inactive against the strains of Proteus.

Indole-positive Proteus. In contrast to *P. mirabilis*, the 58 strains of *P. rettgeri*, *P. vulgaris* and *P. morgani* were markedly resistant to penicillin, as well as most other antibiotics except kanamycin (Fig. 6). Most strains

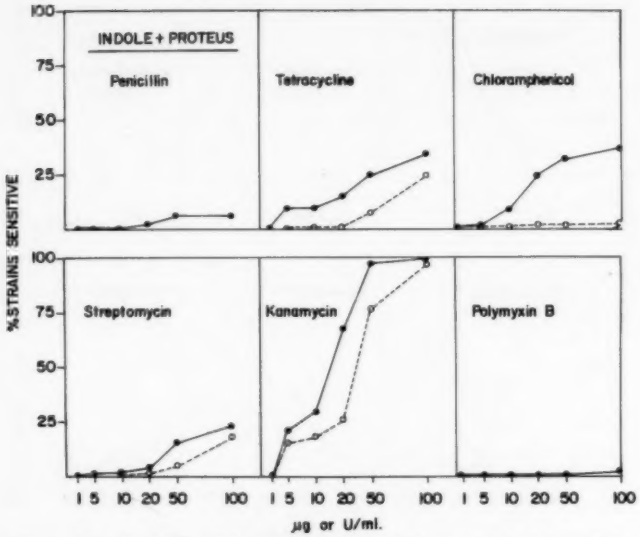

FIG. 6. Bacteriostatic (●—●) and bactericidal (○—○) activity of 6 antibiotics against indole-producing strains of Proteus.

were tested after this agent became available and the data are based upon studies with 42 isolates. Kanamycin at concentrations of 20 microgm. per ml. inhibited two-thirds of the strains and at concentrations of 50 microgm. per ml. was bacteriostatic for 40 of 42 strains. The concentration of drug required for bactericidal effect usually was the same as that required for bacteriostasis.

Other Gram-negative Bacilli. The activity of six antibiotics against 17 strains of certain less common Gram-negative pathogens is depicted in Table II. At clinically-attainable blood levels, *B. anitratum*, *Serratia mar-*

TABLE II

Antimicrobial Activity of 6 Antibiotics Against Certain other Gram-Negative Bacilli

	B. Anitratum	Serratia Marcescens	Achromobacter	Salmonella
No. Strains	5	2	6	6
Penicillin (50 u./ml.)	1*/1†	0/0	0/0	6/4
Tetracycline (5 microgm./ml.)	0/0	0/0	2/1	3/0
Chloramphenicol (10 microgm./ml.)	2/1	0/0	1/1	5/0
Streptomycin (10 microgm./ml.)	1/1	0/0	0/0	0/0
Neomycin (10 microgm./ml.)	0/0	1/0	1/1	1/1
Polymyxin B. (5 microgm./ml.)	0/0	0/0	0/0	0/0

* = Numerator = No. Strains Inhibited.
† = Denominator = No. Strains Killed.

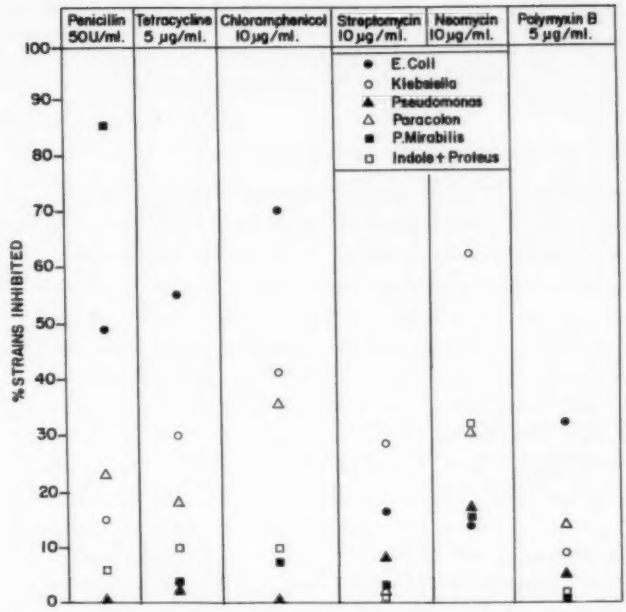

FIG. 7. Comparative bacteriostatic activity of 6 antibiotics against *E. coli*, Klebsiella, Paracolon, Pseudomonas and Proteus species expressed in terms of feasible blood levels.

cescens and Achromobacter were almost totally resistant. At higher levels, neomycin, streptomycin and polymyxin B were capable of considerable antibacterial activity against *B. anitratum* and Achromobacter. The Salmonella were, as might be expected, inhibited by chloramphenicol. Penicillin was also effective in concentration of 50 units per ml. and inhibited all 6 strains four of which were also killed.

Comparative Activity of Antimicrobials against Gram-negative Pathogens

In Figure 7 the antibacterial activity of six antimicrobial drugs is compared. Sensitivity to a concentration of drug that can be attained in the blood during intensive therapy was used as the criterion for comparison. This is admittedly arbitrary and subject to many variables. Nevertheless, employing this approach, it is clear that of the various species tested in this study only *E. coli* and *P. mirabilis* were relatively susceptible to the commonly-used antibiotics. Ten microgm. per ml. of neomycin were capable of inhibiting growth of two-thirds of strains of Klebsiella. Proteus, other than mirabilis, and Pseudomonas were particularly refractory to the action of antibiotics *in vitro*.

Relationship between Site of Isolation and Antibiotic Sensitivity

There was no difference between the pattern of sensitivity of Gram-negative bacilli cultured from urine and strains isolated from blood, sputum, abscesses and body fluids.

DISCUSSION

The importance of Gram-negative bacilli in the pathogenesis of a variety of infections varying from asymptomatic bacteruria to overwhelming sepsis with shock needs no reiteration. Although a large number of variables including bacteremia, associated debilitating disease, existence of purulent foci, prior exposure to chemotherapy, and acquisition of infection in the hospital influence the outcome of infection with these organisms, antimicrobials remain the cornerstone of therapy. Because the need to institute treatment with these drugs often arises before the results of sensitivity tests become known, ability to predict the susceptibility of these pathogens to antibiotics is highly desirable. This is particularly true since there is wide variation in antimicrobial sensitivity among Gram-negative species.

There are, of course, marked differences in sensitivity patterns between individual strains. Nevertheless, studies carried out in many different laboratories by a number of methods demonstrate remarkable similarity in the effectiveness of a given antibiotic against individual species (3, 8, 11 to 14). This is illustrated in Table III in which six commonly-used antibiotics have been ranked according to their bacteriostatic activity. Despite con-

TABLE III

Comparative Effectiveness of 6 Antibiotics Against Gram-Negative Pathogens

	E. Coli					Klebsiella					P. Mirabilis					Other Species of Proteus					Pseudomonas				
	Finland (11)	Kass (8)	Sanford (12)‡	Waisbren (3)	Present Study	Finland (11)	Kass (8)	Sanford (12)	Waisbren (3)	Present Study	Finland (13)	Kass (8)	Sanford (12)	Waisbren (3)	Present Study	Finland (13)	Kass* (8)	Sanford* (12)	Waisbren* (3)	Present Study	Finland (14)	Kass (8)	Sanford (12)	Waisbren (3)	Present Study
Penicillin	5	5	—	6	6	5	5	—	6	6	1	—	—	—	1	5	2	—	2	5	5	5	—	—	6
Streptomycin	4	4	3	5	4	4	3	3	5	4	3	—	—	—	4	4	1	3	5	4	2	2	3	4	4
Tetracycline	3	3	2	2	3	3	4	2	3	5	4	—	—	—	5	1	4	2	4	2	3	3	1	3	3
Chloramphenicol	2	2	1	1	1	2	2	1	2	3	2	—	—	—	2	3	3	1	1	3	4	4	2	5	5
Neomycin	—	—	—	3	5	—	—	—	1	1	—	—	—	—	3	2	—	—	3	1§	—	—	—	2	2
Polymyxin B	1†	1	—	4	2	1	1	—	4	2	5	—	—	—	6	6	5	—	6	6	1	1	—	1	1

* All species of Proteus.

† = Lowest number = Greatest antimicrobial activity.

‡ = Strains obtained in 1953–1954.

§ = Kanamycin.

Number in parentheses = Reference.

siderable differences in the minimum inhibitory concentrations in different laboratories, there is excellent agreement with respect to the relative activity of each agent. The uniformity of these results is even more remarkable in view of the fact that bacterial isolates were obtained under different conditions from a wide variety of foci.

Table III is based upon *in vitro* data on approximately 2000 strains. Chloramphenicol shows the most impressive inhibitory activity against *E. coli*, and is followed closely by polymyxin B. Because less toxic drugs are usually available, polymyxin B is only occasionally indicated in the treatment of *E. coli* infections. The tetracyclines were also effective against many strains of this genus and stand in third place.

The results with Klebsiella were somewhat less consistent but again chloramphenicol was highly effective. As pointed out by Waisbren and Strelitzer (3), neomycin also showed marked antibacterial activity against Klebsiella strains. However, kanamycin displayed the same degree of activity as neomycin (7) and has the advantage that it is less toxic. Polymyxin B and colistin (15) also demonstrated excellent bacteriostatic activity against Klebsiella.

Antimicrobial sensitivity studies with Proteus are only meaningful when *P. mirabilis* is separated from the indole-forming species. This has been pointed out by Finland (13) and has been confirmed in the present study. There is no question that penicillin in relatively high concentration inhibits growth of the vast majority of strains of *P. mirabilis*. Effective concentrations of penicillin can be achieved *in vivo* and this drug has been successfully used in refractory urinary tract infections (16) and bacteremia (17) caused by *P. mirabilis*. In three of the series depicted in Table III, *P. mirabilis* was not differentiated from other Proteus species and the results are therefore less clear-cut. It should be noted, however, that there was excellent agreement between our data and those obtained by Finland (13). The findings with *P. rettgeri*, *P. morgani* and *P. vulgaris* indicate that tetracycline or chloramphenicol should be the first agent employed but that kanamycin should be considered in serious infections with these organisms.

There is little doubt that polymyxin B or colistin are the drugs of choice in Pseudomonas infections, with neomycin, tetracycline and streptomycin following in that order. Chloramphenicol was generally not effective against these strains.

Although streptomycin did not rank highly in any of the groups, a number of isolates was inhibited by this agent. Its bactericidal action and relative lack of toxicity indicate that it should be used whenever sensitivity studies show the offending pathogen to be susceptible.

SUMMARY

The antimicrobial sensitivities of Gram-negative bacteria isolated from patients with clinical infection were assayed against penicillin, streptomycin, tetracycline, chloramphenicol, neomycin and polymyxin B. For some strains tests were also performed with novobiocin, kanamycin and colistin. Both bacteriostatic and bactericidal activity was determined. The results are interpreted from the point of view of the clinical usefulness of these drugs. Chloramphenicol appeared to be the most effective antibiotic against *E. coli*, kanamycin or neomycin against Klebsiella, polymyxin B against Pseudomonas and penicillin against *Proteus mirabilis*.

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FURTHER OBSERVATIONS ON THE DETERMINATION OF THE ISONIAZID INACTIVATOR PHENOTYPE

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INTRODUCTION

It has been shown that there is a genetically determined polymorphism for the inactivation of isoniazid in man. Slow inactivation of the compound is a Mendelian recessive character while rapid inactviation is a dominant character (1 to 3). Previous studies have also shown that there is difficulty in phenotyping a small proportion of individuals with the microbiological technique in common use, and to a lesser extent with a biochemical assay procedure (4).

The plasma isoniazid concentration determined biochemically six hours after ingestion of 10 mg. isoniazid per kg. body weight, has been shown to be correlated with body weight (2). Heavy subjects had significantly higher plasma isoniazid concentrations than light subjects. This relationship was true particularly for slow inactivators, and also to a lesser extent for rapid inactivators of the drug. It suggested firstly that body weight was not an ideal basis for dosage, and secondly that if a better criterion could be found, the separation of the two phenotypes might be improved, especially in light-weight subjects. Therefore, a second series of subjects was tested using a dosage of 40 mg. isoniazid per kg. of "metabolically active mass" (= $\text{Weight}^{0.7}$) (5) and measuring the plasma isoniazid concentration biochemically six hours after drug ingestion.

This communication presents the results from both series of subjects. The results indicate that the "metabolically active mass" is a more suitable basis than body weight, for computation of drug dosages in future pharmacogenetic investigations.

METHODS

Procedure with Human Subjects. Subjects were weighed and the dosages found by means of the two schedules shown in the Appendix. The isoniazid tablets were given orally to fasting subjects. A light meal of toast and coffee was allowed one hour thereafter. Blood was drawn 6 hours after drug ingestion. One-tenth ml. of 1/1000 heparin was used as an anticoagulant for each 10 ml. of blood. The blood sample was kept in a refrigerator and the plasma separated the same day. The plasma was stored at −20° C. until the analysis was performed.

Plasma isoniazid determination. In the series of subjects given a dosage of 10 mg. isoniazid per kg. body weight the plasma isoniazid determinations were carried out using a slight modification of the method of Maher *et al.* (4, 6). The modification consisted in using 4.5 ml. of 0.1N HCl instead of 4.0 ml. This made recovery of 3.0 ml. of the acid layer later in the procedure rather easier.

For the series of subjects given 40 mg. isoniazid per kg. of metabolically active mass, this modification was not employed.

All specimens were read against distilled water at 365 mμ in a Beckman DU spectrophotometer. In each set of determinations standards in duplicate at 0, 1, 2, 3, 4, and 5 microgm. isonazid per ml. in pooled plasma were included. The regression line of optical density (y) upon isoniazid concentration (x) was calculated for these standards in each determination procedure. From this line the standard error of estimation was given by the expression:

$$\frac{\text{Standard deviation of scatter of points about the line}}{\text{Regression coefficient}}$$

The mean value for this expression from 55 lines from the first series of determinations was 0.224 microgm. isoniazid per ml. of plasma. For the second series the mean value derived from 17 lines was 0.220 microgm. isoniazid per ml. of plasma.

The isoniazid concentration in unknowns was computed from their optical density by means of the regression equation obtained in the same determination procedure.

Statistical Methods. Standard methods were used to compute regressions (7). The standard deviation of scatter of points about the line S_r (in units of y) was computed by the following formula (8):

$$S_r = \sqrt{\frac{\Sigma(y - \bar{y})^2 - b^2 \Sigma (x - \bar{x})^2}{n - 2}} \text{ units of } y$$

where: Σ = the sum of; $\bar{x}$ = mean value of x; $\bar{y}$ = mean value of y; n = number of observations; b = regression coefficient

RESULTS

Four hundred four subjects were studied using the 10 mg. isoniazid per kg. body weight dosage. The distribution of their six-hour plasma isoniazid concentrations is definitely bimodal with the antimode at 2.50 microgm. per ml. concentration. Subjects with concentrations below this value are termed rapid inactivators, while those with higher concentrations are termed slow inactivators (Fig. 1A).

Two hundred twenty subjects were studied using a dosage of 40 mg. isoniazid per kg. of metabolically active mass (MAM = $\text{Wt.}^{0.7}$) (5). The six-hour plasma isoniazid values are again markedly bimodal. The antimode is at 3.75 microgm. per ml. concentration (Fig. 1B).

Scattergrams of the plasma isoniazid concentrations plotted against the weights of the subjects are shown in Figures 2 and 3. The regression lines in these figures are those whose parameters are shown in Table I.

As will be seen from Table I there is a highly significant slope to the

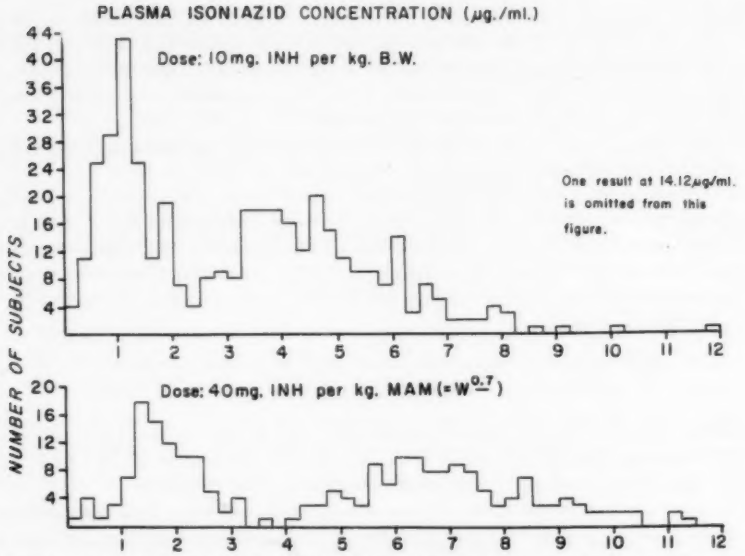
PLASMA ISONIAZID CONCENTRATION (μg./ml.)
Dose: 10mg. INH per kg. B.W.
One result at 14.12μg/ml. is omitted from this figure.
NUMBER OF SUBJECTS
Dose: 40mg. INH per kg. MAM (=W^0.7)

FIG. 1A. (Above) The distribution of plasma isoniazid concentrations six hours after drug ingestion. Dosage schedule = 10 mg. isoniazid per kg. body weight.

FIG. 1B. (Below) The distribution of plasma isoniazid concentrations six hours after drug ingestion. Dosage schedule = 40 mg. isoniazid per kg. metabolically active mass (Body Weight$^{0.7}$).

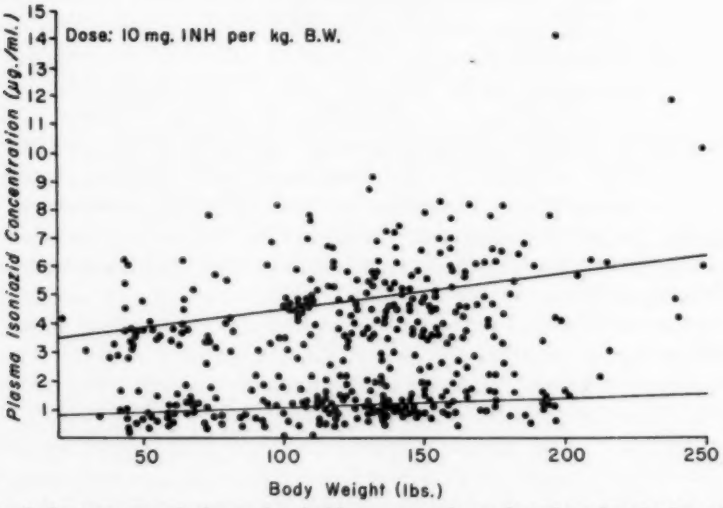

FIG. 2. Scattergram of plasma isoniazid concentrations plotted against body weight. Dosage schedule = 10 mg. isoniazid per kg. body weight. The parameters of the regression lines are given in Table I.

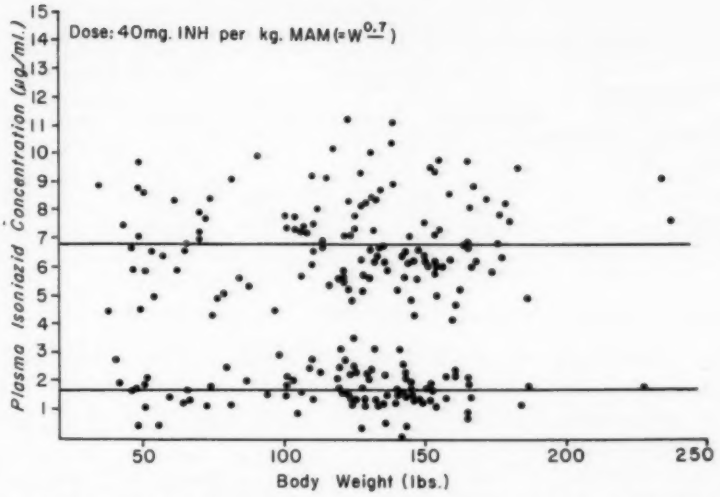

FIG. 3. Scattergram of plasma isoniazid concentrations plotted against body weight. Dosage schedule = 40 mg. isoniazid per kg. metabolically active mass (Body Weight$^{0.7}$).

TABLE I

Parameters of Regression of Plasma Isoniazid Concentration (y) upon Weight (lbs.) (x)

Parameter of Regression	Dose: 10 mg. INH per kg. BW		Dose: 40 mg. INH per kg. MAM	
	Slow Inactivators	Rapid Inactivators	Slow Inactivators	Rapid Inactivators
Number of pairs of observations n	224	180	128	92
Correlation Coefficient r	0.32696	0.28343	0.03415	0.000316
Significance of r	$p < 0.001$	$p < 0.01$	$p > 0.10$	$p > 0.10$
Regression Coefficient b	0.011623	0.003449	0.001302	0.000018
Intercept a	3.349	0.735	6.840	1.752
Standard deviation of scatter of points about the line S_y (in units of y)	1.5403	0.5185	1.5502	0.6747
Standard Error of b	0.002255	0.000874	0.003395	0.001917
b/SE_b	5.15432	3.94374	0.383506	0.009473
Significance of b	$p < 0.001$	$p < 0.001$	$p > 0.10$	$p > 0.10$

regression of plasma isoniazid upon weight when the dosage employed was 10 mg. per kg. body weight. There is, however, no significant correlation or regression between the two variables when the dosage was 40 mg. per kg. metabolically active mass.

The distribution of weights (Table II) shows that there is no significant difference between the weight distributions of the different phenotypes or the populations tested by the two dosage schedules.

TABLE II

The Distributions of the Body Weights (lbs.) of the Experimental Subjects Studies

	Dose: 10 mg. INH/kg. BW		Dose 40 mg. INH/kg. MAM	
	Slow Inactivator	Rapid Inactivator	Slow Inactivator	Rapid Inactivator
Number	224	180	128	92
Mean	126.8170	121.7556	123.6641	121.4131
SD	45.74	44.316	40.362	36.906
SE of Mean	3.056	3.303	3.567	3.848

DISCUSSION

The series of patients studied using a dosage of 10 mg. per kg. body weight shows a significant relationship between plasma isoniazid concentration and weight. This fact could lead to misclassification of the phenotype of light-weight subjects.

This relationship is entirely annulled when a dosage of 40 mg. per kg. metabolically active mass (MAM = $Wt.^{0.7}$) (5) is used. The distribution curves of the plasma isoniazid concentrations shows a clearer separation of the two phenotypes at this dosage than with a dosage of 10 mg. per kg. body weight. The mean weight of the 624 individuals studied is 124 lbs. (= 56 kg.) and at this weight 10 mg. per kg. BW gives a dose of 560 mg. For the same body weight the metabolically active mass ($BW^{0.7}$) is 16.74 kg. and a dosage of 40 mg. per kg. MAM gives a dose of 670 mg. The greater separation of the two curves on this latter dosage schedule is therefore due to a 20 per cent increase in dose for the average subject, as well as to its calculation in terms of a different body parameter.

The phenotypes are determined by a pair of autosomal allelic genes, rapid inactivation being a dominant character (2, 3). A "dosage" effect is demonstrable, in that subjects homozygous for the allele controlling the dominant character have on the average, lower plasma isoniazid levels than heterozygotes (2, 3). It is seen that the increased dosage of 40 mg. per kg. MAM has resulted in a significant raising of the mean plasma isoniazid level of the rapid inactivators (Table III). It may be speculated that this represents the effect of an increased substrate load, particularly in the case of the heterozygotes which constitute 80 per cent of the dominants in European and American Negro populations. The raising of the level in slow inactivators (Table III) presumably shows the effect of an increased substrate load on other isoniazid-removing mechanisms in the body.

These results indicate two principles to be applied in future pharmacogenetic investigations. Firstly, drug doses should be assigned in terms of metabolically active mass. Secondly, dosages should be large to clearly sep-

TABLE III

The Distributions of the Plasma Isoniazid Concentrations (μg./ml.) Six Hours Following Drug Ingestion

	Dose: 10 mg. INH/kg. BW		Dose: 40 mg. INH/kg. MAM	
	Slow Inactivator	Rapid Inactivator	Slow Inactivator	Rapid Inactivator
Number	224	180	128	92
Mean	4.823	1.155	7.011	1.754
SD	1.626	0.539	1.545	0.671
SE of Mean	0.1087	0.0402	0.1365	0.0699

arate individuals who possess a genetically conferred enzymic mechanism from those in whom it is absent.

SUMMARY

There is a genetically determined polymorphism for the inactivation of isoniazid in man. Determination of the phenotype is carried out by estimating the plasma isoniazid concentration six hours following drug ingestion.

A significant correlation was found between plasma isoniazid concentration and weight when a dosage scheme of 10 mg. isoniazid per kg. body weight was used. Heavier subjects had higher concentrations than light subjects. Difficulty was experienced in accurately phenotyping a small proportion of subjects.

When a dosage scheme of 40 mg. isoniazid per kg. metabolically active mass (= Body Weight$^{0.7}$) was used, the correlation disappeared and the separation of the two phenotypes became more distinct.

It is suggested therefore that the employment of a large dose of drug computed on the basis of metabolically active mass is an important principle for future pharmacogenetic investigations.

ACKNOWLEDGMENTS

The authors wish to acknowledge the help given them by the following persons: Mrs. Barbara Mitchell, Miss Margaret Hawkins, and Mrs. Dorothy Chilcoat for assistance in the study of test subjects; Dr. Carmen F. Merryman and Mrs. Linda Ulenurm for technical assistance.

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APPENDIX

Dosage Schedule 10 mg. Isoniazid per kg. Body Weight

Weight (lbs.)	Dose INH (mg.)	Weight (lbs.)	Dose INH (mg.)
32–36	150	118–129	550
37–42	175	130–140	600
43–48	200	141–151	650
49–53	225	152–162	700
54–59	250	163–174	750
60–65	275	175–185	800
66–70	300	186–196	850
71–74	325	197–208	900
75–84	350	209–218	950
85–95	400	219–230	1000
96–106	450	231 +	1050
107–117	500		

Dosage Schedule 10 mg. Isoniazid per kg. Metabolically Active Mass

Weight (lbs.)	Dose INH (mg.)	Weight (lbs.)	Dose INH (mg)
32–37	275	122–128	675
38–41	300	129–135	700
42–46	325	136–142	725
47–51	350	143–148	750
52–56	375	149–155	775
57–62	400	156–162	800
63–67	425	163–170	825
68–72	450	171–177	850
73–78	475	178–186	875
79–84	500	187–194	900
85–90	525	195–199	925
91–96	550	200–207	950
97–102	575	208–214	975
103–108	600	215–222	1000
109–115	625	223–230	1025
116–121	650	231–238	1050
		239–246	1075

ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

October 10, 1960

IDIOPATHIC HYPERGLYCINEMIA, A NEW DISORDER OF AMINO ACID METABOLISM

WILLIAM L. NYHAN, BARTON CHILDS, AND ROBERT E. COOKE

A new disorder of amino acid metabolism, manifested by abnormal glycinemia and glycinuria has been observed in a patient studied in the Harriet Lane Home. The onset of illness was at 18 hours of life with vomiting, acidosis and ketosis, and these manifestations have persisted among the cardinal features of disease. In the first months of life he was noted to have neutropenia, thrombocytopenia, and occasional episodes of purpura. Frequent infections were associated with a low level of circulating γ-globulin. It became evident early that he was developmentally retarded, and the developmental quotient has ranged, over the three years of his life to date, from 40 to 50. From 3 to 6 months of age he had occasional seizures, and electroencephalography revealed a seizure pattern. More recently there have been no seizures, and the EEG has been normal, but, with episodes of acute illness, slow, aimless movements of the extremities have been observed, which are similar to athetoid movements but finer. His skull is long and narrow, but no other abnormalities of the skull could be seen on physical or roentgenographic examination. X-rays of the long bones revealed marked osteoporosis. Examination of the urine by paper chromatography revealed an intense glycine spot.

Quantitative estimation of glycine content documented an intense glycinuria and indicated further that plasma glycine levels in this patient approximated ten times those of controls. The adequacy of a number of pathways of glycine metabolism has been assessed by comparison of the levels of certain products with those of suitable control individuals. These included the pathway through δ-aminolevulinic acid to hemoglobin, through glyoxylate to oxalate, and pathways to glutathione, creatine and creatinine. Conjugation with benzoate and salicylate was performed normally, and the usual amounts of bound glycine were released by acid hydrolysis of the urine. The incorporation of glycine into protein, and its conversion to CO_2 and various intermediates were studied using C^{14}-labeled glycine which was added in Warburg flasks to slices and homogenates of the patient's liver. These experiments are still in progress, but major deviations from normal in the utilization of glycine have not been observed. On the other hand, oral loads of glycine were followed by marked elevations of plasma glycine which were abnormally prolonged. Changes in serine levels could be correlated with these changes in glycine levels, and similarly the administration of serine was followed by elevations of glycine in blood and urine. Labeled serine was isolated in the experiments noted in which C^{14}-labeled glycine was the tracer.

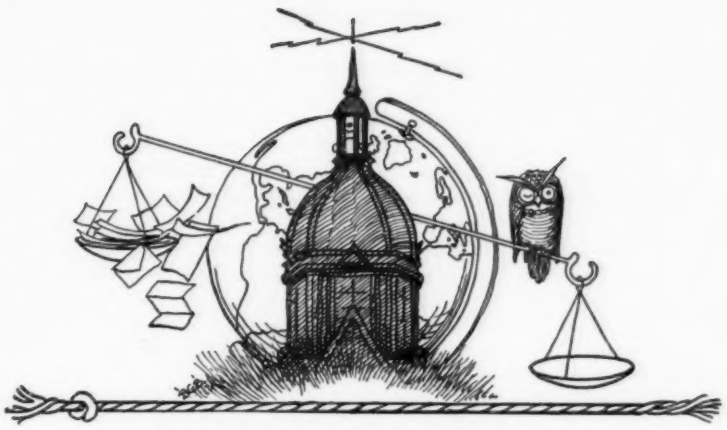
In the course of these experiments it has been found that the acute episodes of ketosis and metabolic acidosis were related to the amount of protein or amino acids in the diet, and reduction of the dietary intake of protein has reduced the frequency and severity of these attacks. Concomitantly the concentrations of glycine in blood and urine have decreased and the neutropenia has improved. Thrombocytopenia dis-

appeared spontaneously before dietary regulation. Marked osteoporosis and developmental retardation have persisted without apparent change. Lowering of glycine levels alone by the administration of benzoate did not prevent the production of typical metabolic acidosis by the administration of a large dietary load of protein. Some of the symptoms of illness could be reproduced by mixtures of amino acids containing leucine. Investigation of the concentrations of amino acids of plasma and urine using chromatography on cation exchange columns revealed elevations of serine, alanine, isoleucine and valine in the plasma, which were however not as great as that of glycine. Leucine tolerance tests in the patient were distinctly abnormal, while the administration of oral loads of leucine was found to induce a fall in the plasma levels of a number of amino acids in both patient and controls. The data described in this abstract will be published.

PNEUMOCYSTIS CARINII INFECTION

WALTER H. SHELDON

The findings in several North American cases of pulmonary pneumocystis infection were described. The disorder was observed either as massive pneumonia or as a subclinical pneumonitis in several children, one teen-age girl and in two adults. These observations and those of other instances in this country were compared with the findings recorded in the European literature. The results of the experimental production of the infection in rabbits were reported. It appears that in this country latent pulmonary infection with this agent is probably not uncommon in man and in a variety of animals and it was concluded that clinically manifest disease is a manifestation of a preceding severe impairment of host resistance.



BOOK REVIEWS

Fundamentals of Chest Roentgenology. By BENJAMIN FELSON. 301 pp., $10.00. W. B. Saunders Co., Philadelphia, Pa., 1960.

This is a rather small book on a very large subject. Yet when the material is examined, apologies of the author for brevity and shortcomings notwithstanding, it must be admitted that justice has been done to the subject of chest roentgenology. This is due to the fundamental nature of the material treated. The author restricts himself to a basic physical and anatomical approach to disease of the chest from the x-ray standpoint, but this is done with such thoroughness and elegance that the entire gamut of roentgen manifestations is extremely well covered.

In his approach the author considers chest disease from the topographic standpoint, discussing radiographic manifestations of diseases of the pulmonary lobes and segments, the hila, the pleura, the extrapleural space, and the diaphragm. The text is extremely readable due to the fact that the author writes fluently on the basis of his own experience, evidently without the customary mechanical gathering of material from the literature, and inserting it in the appropriate section of the text. This is not to say that the author has not made adequate reference to the literaure. He has done so, and there is an excellent bibliography. It is simply to emphasize the rather unique continuum of thought and approach which characterize this work. It might be compared in its relationship to the radiologic literature (with reference to thoracic radiology) to Walshe's classic text on "Diseases of the Nervous System" in relation to the field of clinical neurology.

Because of the basic, though unhackneyed, nature of this presentation, because of the skill with which the material to illustrate the principles of the text is selected and because of the evident scrupulous integrity with which the author attacks his subject, this book is enthusiastically recommended to all who are interested in chest disease.

DANIEL TORRANCE, JR.

Electrical Studies on the Unanesthetized Brain. A Symposium. Edited by ESTELLE R. RAMEY and DESMOND S. O'DOHERTY. 423 pp., $6.00. Paul B. Hoeber, Medical Book Dept. of Harper & Bros., New York, N. Y., 1960.

In the spring of 1957 a three day conference was held at Georgetown University on depth recording. The papers given at that conference and the stenographic transcrip-

tions of the discussion provide the basis for this book. At the conference, both animal and human recording and stimulation were presented. The depth stimulation in animals emphasized the reward systems or start and stop systems which have been revealed in the brain during the last decade. Subcortical recording in patients with epilepsy, involuntary movements and psychosis has been used in a number of centers; the results are presented by several authors who give a well rounded account of their findings.

There are adequate references to current literature at the end of each chapter; the book has a good index. The entire volume is well printed and beautifully illustrated. It gives a well edited account of one of the first symposiums on this subject. Even though the book is somewhat late in appearing, it will be welcomed by all interested in the functions of the nervous system for the techniques described offer a new and exciting means of revealing certain mechanisms of the brain.

A. Earl Walker

Insulin. British Medical Bulletin, Vol. 16, No. 3, Sept. 1960. Edited by Professor F. G. Young. 264 pp., $3.25. Published by the Medical Department, The British Council, London W.1, England.

We have nothing in the United States to match the uniform excellence of the symposia offered by the British Medical Bulletin. This one, on insulin, is especially rewarding.

F. G. Young, in his introduction, says of insulin that "its clinical value fortunately in no way depends upon an understanding of its mechanism of action. Indeed, when one considers the multitude of scientific papers that have been published since the discovery of insulin forty years ago, the uncertainty of knowledge about the mechanism of action of this hormone is surprising. Whether or not this substance has a single point of action or acts upon different processes is far from clear... The fact that insulin acts on carbohydrate metabolism is clearly shown by its potent effect in abolishing hyperglycaemia and glycosuria in diabetes... The actions of insulin upon fat metabolism and upon protein metabolism are perhaps no less important though less obvious."

And this is the theme of the symposium, supplemented by reviews of the use of insulin in clinical practice and the chemistry and application of other hypoglycemic substances. All of the subjects are well covered by men who bring to their reviews critical analysis based on their rich experience in the field. So rapidly is work on insulin proceeding, however, that one is aware of perhaps half a dozen significant papers which have appeared in the past year and are not mentiond in the Bulletin. One whole arena of insulin action escapes without notice. The fact that insulin reduces the concentration of serum potassium is exploited clinically for management of hyperkalemia. In many ways, the effect of insulin on potassium metabolism is quantitatively greater than any of its other known effects and forms the basis for a body of investigation into the mechanism of action of insulin. It is curious that the role of insulin in mineral metabolism has been ignored in this symposium, but certainly no other fault can be found.

Kenneth L. Zierler

The Art of Marriage Counseling. A Modern Approach. By W. L. Herbert and F. V. Jarvis. 125 pp., $2.75. Emerson Books, New York, N. Y., 1960.

The small size of this book may be misleading to persons unfamiliar with the in-

tricate problems indigenous to the process of marriage counseling. Such readers might conclude further that the equally deceptive simplicity of writing style signifies only limited grasp of the subject matter. However, practitioners of marriage counseling, whether representing medicine, social work, the law, clergy, or others, will be impressed with the profound nature of what this book sets out to do.

The authors address themselves to the problem of helping married couples within the Western norm of monogamy to adjust themselves more acceptably to the marital situation. They trace the interaction of personality factors, societal pressures, and the establishment of an effective counseling role as these are interdependent and complementary while coming to focus, as they must, on the rehabilitation of a marriage relationship. This is no easy task, but the degree of success these authors achieved in approaching it was impressive.

The greatest value of this book, therefore, appears to be the high potential in it for assisting practitioners in the field of marriage counseling to refurbish their skills and techniques. Periodically, this is essential for successful practice. The chapters concerning the interview and its development, although leaning toward social casework as the major source of information, are most informative for practitioners from other disciplines.

Despite the emphasis on an underlying, guiding philosophy, it was refreshing to see practical aspects of case recording and case consultations discussed in terms of their usefulness in practice. Also, the case illustrations are pertinently formulated, lending weight to the related discussions concerning the rationale for methods used by the counselor in handling them.

The role of unconscious motivation was emphasized throughout with good effect. This was discussed as applying to both the counselor and his "client" (a word which the authors acknowledged to be inadequate, but which they used because there doesn't seem to be a better one available). How the two sets of unconscious motivations may interact, both for positive and negative aspects of counseling was made quite clear.

One point that is only mentioned briefly is the problem of keeping from taking sides with one marital partner against the other. This is a particularly difficult aspect of marriage counseling and one to which the counselor must be constantly alert. Also, a discussion which could have been elaborated to some extent concerns the theoretical implications relating to transference problems as they may be influenced during counseling sessions where both partners are seen together. Finally, the premise that a marriage has to be defined as being unhappy before counseling is indicated may be misleading. It is possible that marriages which are fairly well integrated can also benefit from brief counseling contact. This concept might be extended to how the relatively happily married couple may use the counselor as a consultant on a one or two-session basis.

It would be interesting to see these authors focus their unusually clear insights and lucid writing more fully on topics such as these in some future publication.

ANTHONY R. STONE

Delayed Effects of Whole-Body Radiation: A Symposium. Jointly sponsored by the Operations Research Office and the Walter Reed Army Institute of Research. Edited by BERNARD B. WATSON. 80 pp., $4.50. Published for Operations Research Office, The Johns Hopkins University by The Johns Hopkins Press, Baltimore, Md., 1960.

From industrial and military accidents, from clinical experience and from study of

the war-time nuclear explosions in Japan, the immediate effects of exposure to nuclear radiations are reasonably well known but the knowledge of the late effects of such exposure is based at the present time largely on animal experimentation and extrapolation to the human race can lead to no more than interesting speculation. In the event of a nuclear war, military personnel would be exposed to levels of radiation considerably above those now considered permissible and in October 1959, the Operations Research Office and the Walter Reed Army Institute of Research held a symposium to summarize what is already known of the delayed effects of whole body irradiation and to provide guidance for future investigation. The papers presented at this symposium together with some of the discussion which followed each paper have now been published in this small volume.

The four main topics for discussion were the effects on life shortening, the induction of leukemia and malignant disease, the production of cataracts, and genetic alterations. On the first three subjects two papers were presented, one summarizing the evidence from animal experimentation and the second the known effects and the speculative deductions from this evidence of the effects in man. In regard to the genetic effects, only one paper was presented, an excellent summary by Dr. Grahn, which summarizes the experimental evidence in animals and the probable inferences which can be drawn from these studies. As for the effects of radiations on longevity, the observed life shortening in animals has been extrapolated to man and at least three different theories and mathematical formulae have been used to calculate the probable shortening of life span in man resulting from exposure to radiation. Whilst there is good agreement between the figures arrived at by these different calculations, it is the conclusion of Dr. Berlin that such extrapolations should not serve as a basis for estimating the hazard to man.

In considering the induction of leukemia and malignancy the symposium was on surer ground. A summary is presented of radiation carcinogenesis and leukemogenesis arising from study of children exposed to therapeutic doses of radiation, or to diagnostic doses in utero, of survivors from the Japanese atomic explosions, and from the calculated increase in leukemia in American radiologists and in patients with spondylitis treated by x-ray therapy. Even with this evidence, however, only two definitive conclusions can be drawn, first that there is an association between irradiation and an increase in leukemia in the higher dose range and that carcinoma of the thyroid is more common in children irradiated for benign disease of the head and neck. The question of a threshold dose in the induction of leukemia is critically examined but insufficient data are available to confirm the existence of such a threshold and the conclusion reached by E. B. Lewis, that each roentgen of irradiation is equally effective in inducing leukemia is open to question.

Ionizing radiations are known to cause cataracts if the lens is exposed and it is perhaps surprising that so little is known of the dosage level required but with the increasing use of neutron irradiation, cataracts would be expected to occur more frequently and this fact may stimulate further investigation in this field. It is concluded that if allowance is made for differential absorption, there is no difference in the dosage of X or gamma radiation required to induce cataracts and this may also be true of ultra-violet and beta irradiation by neutrons are five to ten times as cataractogenic as x-rays. The question as to whether microwaves and ultra high frequency radiation will cause cataract is still under discussion.

This small volume then, provides a definitive summary of the experimental and clinical evidence to date of the long term effects of exposure to radiations. As such,

it is of considerable interest to workers in this field but it is disappointing to find so few definitive suggestions as to the lines along which further research in this most important subject should be guided.

ROBERT J. DICKSON

Fundamentals of Otolaryngology, 3rd edition. By LAWRENCE R. BOIES AND ASSOCIATES. 510 pp., $8.00. W. B. Saunders Co., Philadelphia, Pa., 1959.

Designed primarily for the undergraduate medical student, this book with its logical and non-technical approach to the fundamentals of otolaryngology serves as a splendid reference manual for the non-specialist and general practitioner as well. It covers the modern field of otolaryngology by including adequate discussions of reconstructive nasal surgery, maxillofacial surgery, and tumefactions of the neck. Each subject in otolaryngology is taken up in a very orderly manner beginning with the applied anatomy and physiology of each region and followed by a concise description of how to examine that particular area. The diseases common to that area are then discussed primarily in the order of frequency and importance. Each is followed by a resumé of recommended therapy. Further elaboration on the latter is taken up in a final chapter dealing with a number of the modern drugs and their value to otolaryngology.

M. LEE WILLIAMS

BOOKS RECEIVED FOR REVIEW

Adventure to Motherhood. By J. ALLAN OFFEN. 68 pp., $2.95. Audio Visual Education Co. of America. Distributed exclusively in the U. S. by Taplinger Publishing Co., New York, N. Y., 1960.

Current Medical Research. A reprint of the articles in the Report of the Medical Research Council for the year 1958–59. 56 pp., 3s. 6d. net. Her Majesty's Stationery Office, London, England, 1960.

Biochemical Values in Clinical Medicine. The Results following Pathological or Physiological Change. By ROBERT DUNCAN EASTHAM. 144 pp., $3.75. John Wright & Sons, Bristol, England. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. Agents, 1960.

Care of the Well Baby. Medical Management of the Child from Birth to Two Years of Age. By KENNETH S. SHEPARD. 224 pp., $3.25. J. B. Lippincott Co., Philadelphia, Pa., 1960.

Ciba Foundation Colloquia on Endocrinology, Vol. 13. Human Pituitary Hormones. Edited by G. E. W. WOLSTENHOLME AND CECILIA M. O'CONNOR. 336 pp., $9.50. Little, Brown & Co., Boston, Mass., 1960.

Ciba Foundation Symposium on Congenital Malformations. Edited by G. E. W. WOLSTENHOLME AND CECILIA M. O'CONNOR. 308 pp., $9.00. Little, Brown & Co., Boston, Mass., 1960.

Hypnosis in Treatment. By WILLIAM MOODIE. 168 pp., $4.00. Emerson Books, New York, N. Y., 1960.

Man and His Body. The Wonders of the Human Mechanism. By BENJAMIN F. MILLER AND RUTH GOODE. 376 pp., $5.95. Simon and Schuster, New York, N. Y., 1960.

The Normal Skull. By ROBERT SHAPIRO AND ARNOLD H. JANZEN. 257 pp., $18.00. Paul B. Hoeber, Medical Book Dept. of Harper & Bros., New York, N. Y., 1960.

Pharmacology in Nursing, 8th edition. By ELSIE E. KRUG. 805 pp., $6.00. The C. V. Mosby Co., St. Louis, Mo., 1960.

Principles of Human Genetics, 2nd edition. By CURT STERN. 753 pp., $9.50. W. H. Freeman & Co., San Francisco, Cal., 1960.

Progress in Experimental Tumor Research. Edited by F. HOMBURGER. 476 pp., $22.00. Published in Basel, Switzerland by S. Karger. Published and distributed in North America by J. B. Lippincott Co., Philadelphia, Pa., 1960.

Protein and Amino Acid Requirements in Early Life. By L. EMMETT HOLT, JR., PAUL GYÖRGY, EDWARD L. PRATT, SELMA E. SNYDERMAN AND WILLIAM M. WALLACE. 63 pp., $1.00. New York University Press, New York, N. Y., 1960.

Staining Methods. Histologic and Histochemical. By J. F. A. MCMANUS AND ROBERT W. MOWRY. 423 pp., $10.00. Paul B. Hoeber, Medical Book Dept. of Harper & Bros., New York, N. Y., 1960.

Treatment of Cancer and Allied Diseases, 2nd edition, Vol. 4, Tumors of the Breast, Chest and Esophagus. Edited by GEORGE T. PACK AND IRVING M. ARIEL. 667 pp., $30.00. Paul B. Hoeber, Medical Book Dept. of Harper & Bros., New York, N. Y., 1960.

HOPKINS-2-HEMOGLOBIN: A REVISED PEDIGREE WITH DATA ON BLOOD AND SERUM GROUPS

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Received for publication August 29, 1960

In 1958 Smith and Torbert (1) described a new hemoglobin, Hopkins-2 (Ho-2). The Fuller kindred, in which this abnormality appeared, also exhibited segregation of the allele for sickle cell hemoglobin (S) as shown in Figure 1. The observed pattern of inheritance suggested that hemoglobin synthesis is controlled by at least two genetic loci. At about this same time hemoglobin was shown to consist of two species of polypeptide chains (2) designated α and β. Evidence that in this kindred Ho-2 represents a lesion of the α chain and S a β chain lesion is described by Itano and Robinson in another report (3).

The purpose of this communication is to present an amended pedigree of the Fuller kindred (Fig. 1) and to record blood and serum group data. Independent inheritance of the genes for Hopkins-2 and sickle cell hemoglobin, and thereby of α and β polypeptide chains, is established beyond reasonable doubt.

METHODS

The hemoglobin of every individual except II-7 was re-examined by paper (4) and agar gel (5) electrophoresis. Serum haptoglobins were examined by starch gel electrophoresis (6). Serum gamma globulin groups were determined by the method of Harboe (7).

RESULTS

Most of the data are shown in Figure 1. I-2 was evidently type B, *MS.Ns, Cc.* His daughter II-4 must be *A.O.* For convenience the MN data are shown as genotypes, ignoring only the possibility of genes MS^u or NS^u: no assumptions were made that could affect the validity of the linkage counts. Most persons were tested with anti-s. The Rh data are given in the phenotype notation of Wiener. All were tested for Fy^a and only III-7 was Fy(a+). All A's were of subgroup A_1. All were negative with anti-C^w, anti-K, anti-Lu , anti-Vw, anti-Wr^a, and anti-Kp^a. All were positive with anti-e and anti-Kp^b. Serum transferrin was type CC in every instance. All were Gm(a+ b +) except III-11 who was Gm(a + b −).

Considering only "certain" families and assuming no illegitimacies, linkage counts were obtained as shown in Table I. A "certain" family is

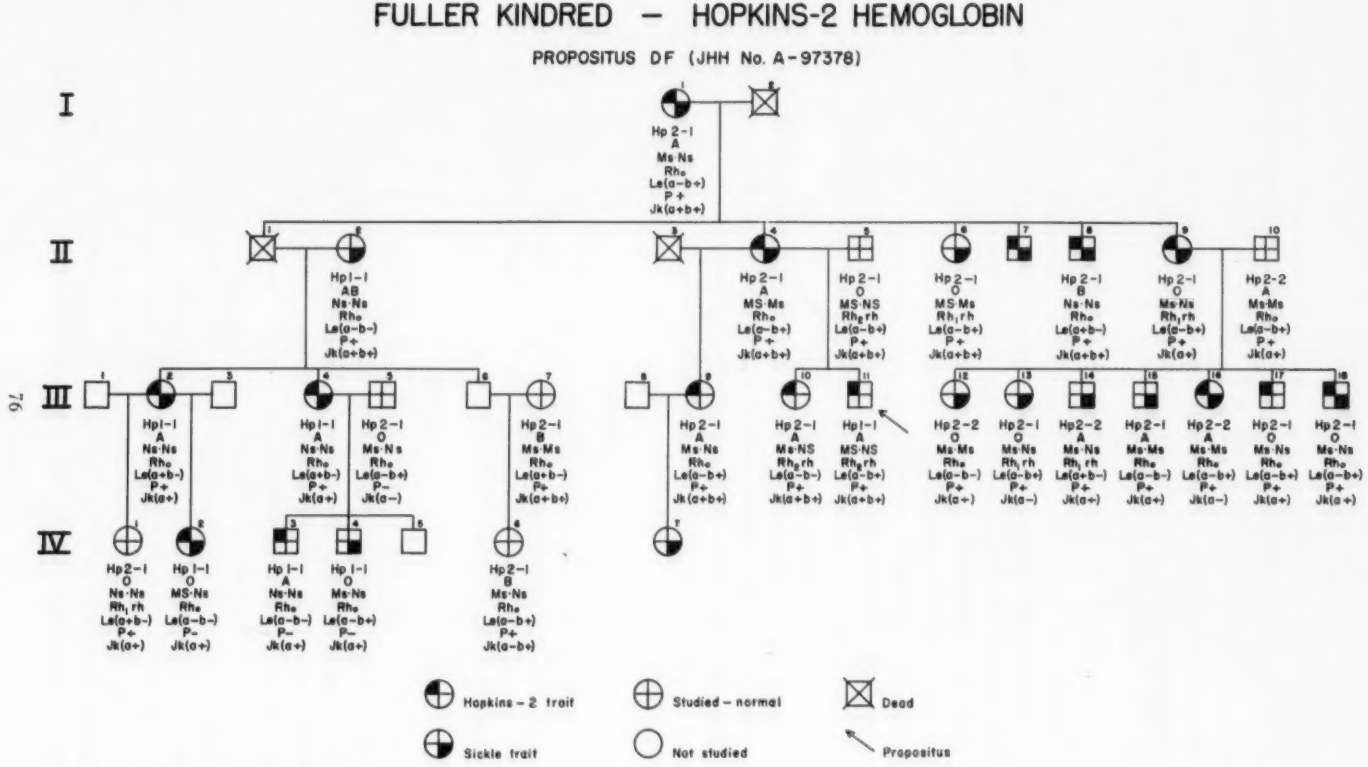
FULLER KINDRED — HOPKINS-2 HEMOGLOBIN
PROPOSITUS DF (JHH No. A-97378)
I
II
III
IV
Hp 2-1 A Ms·Ns Rho Le(a−b+) P+ Jk(a+b+)
Hp1-1 AB Ns·Ns Rho Le(a−b−) P+ Jk(a+b+)
Hp 2-1 A MS·Ms Rho Le(a−b+) P+ Jk(a+b+)
Hp 2-1 O MS·NS Rhgrh Le(a−b+) P+ Jk(a+b+)
Hp 2-1 O MS·Ms Rh1rh Le(a−b+) P+ Jk(a+b+)
Hp 2-1 B Ns·Ns Rho Le(a+b−) P+ Jk(a+b+)
Hp 2-1 O Ms·Ns Rh1rh Le(a−b+) P+ Jk(a+)
Hp 2-2 A Ms·Ms Rho Le(a−b+) P+ Jk(a+)
Hp1-1 A Ns·Ns Rho Le(a+b−) P+ Jk(a+)
Hp 2-1 O Ms·Ns Rho Le(a−b+) P− Jk(a−)
Hp 2-1 B Ms·Ms Rho Le(a+b−) P+ Jk(a+b+)
Hp 2-1 A Ms·Ns Rho Le(a−b+) P+ Jk(a+b+)
Hp 2-1 A Ms·NS Rhgrh Le(a−b−) P+ Jk(a+b+)
Hp1-1 A MS·NS Rhgrh Le(a−b+) P+ Jk(a+b+)
Hp 2-2 O Ms·Ms Rho Le(a−b−) P+ Jk(a+)
Hp 2-1 O Ms·Ns Rh1rh Le(a−b+) P+ Jk(a−)
Hp 2-2 A Ms·Ns Rh1rh Le(a+b−) P+ Jk(a+)
Hp 2-1 A Ms·Ms Rho Le(a−b−) P+ Jk(a+)
Hp 2-2 A Ms·Ms Rho Le(a−b+) P+ Jk(a−)
Hp 2-1 O Ms·Ns Rho Le(a−b+) P+ Jk(a+)
Hp 2-1 O Ms·Ns Rho Le(a−b+) P+ Jk(a+)
Hp2-1 O Ns·Ns Rh1rh Le(a+b−) P+ Jk(a+)
Hp 1-1 O MS·Ns Rho Le(a−b−) P− Jk(a+)
Hp 1-1 A Ns·Ns Rho Le(a−b−) P− Jk(a+)
Hp 1-1 O Ms·Ns Rho Le(a−b+) P− Jk(a+)
Hp 2-1 B Ms·Ns Rho Le(a−b+) P+ Jk(a−b+)
Hopkins – 2 trait
Sickle trait
Studied – normal
Not studied
Dead
Propositus

FIG. 1. Pedigree of Fuller Kindred.

TABLE I

Linkage Counts on Fuller Kindred

	Kidd	P	Lewis	Rh	MN	ABO	Haptoglobin	Sickle hemoglobin
Hopkins-2-hemoglobin	1:1	1:1	3:0	5:2	4:3 1:1	1:1 2:0 2:0	4:3	5:2 2:0 2:0
Sickle hemoglobin	2:0	1:1	3:0	4:3	4:3 2:1	2:0 2:0	4:3	
Haptoglobin	1:1	—	2:1 1:1	4:3	5:2 1:1	—		
ABO	1:1	1:1	2:1	3:1	3:1 2:2 1:1			
MN	1:1	—	2:1 2:0	5:2 2:2 2:0				
Rh	1:1	—	2:1					

defined as one in which it is absolutely clear which gene has been inherited from each parent. The gene producing Hopkins-2 hemoglobin is considered a dominant, as are those producing sickle hemoglobin, haptoglobin-1, haptoglobin-2, and the Lewis factor Le^b. Ratios shown are non-recombinations : recombinations.

DISCUSSION

The revised pedigree differs from that presented by Smith and Torbert by the addition of individuals descending from II-1 and II-2 and by changes among the children of II-9 and II-10.

The present pedigree strengthens the conclusion of the original authors, viz., hemoglobin synthesis is controlled by at least two genetic loci. There is no evidence for close linkage between the loci governing Ho-2 and S hemoglobins. Within the Fuller kindred three doubly heterozygous individuals (II-4, II-9, and III-4), i.e., individuals with hemoglobins Ho-2 and S in addition to adult hemoglobin[1] A, have had children by spouses known

[1] The "normal" component in the doubly heterozygous individuals has been shown by Itano and Robinson (3) to contain two hemoglobins with identical electrophoretic migration characteristics: adult hemoglobin ($\alpha_2^A\beta_2^A$) and a hemoglobin with both α and β chain abnormalities ($\alpha_2^{Ho\text{-}2}\beta_2^S$).

to have entirely hemoglobin A. Eleven children of these matings have been studied and consist of

Four (III-10, III-11, III-17 and IV-3) with hemoglobins Ho-2 and A,
Five (III-12, III-13, III-14, III-15 and IV-4) with hemoglobins S and A, and
Two (III-16 and III-18) with hemoglobins Ho-2, S, and A.

In addition, a doubly affected woman, III-2, has a normal daughter, IV-1. The father's hemoglobin type is unknown but, in any case, it is in this instance irrelevant. The numbers are small and no exact test of agreement with a 1:1:1:1 expectation can be applied. What is notable is that all four phenotypes are present.

Since the genetic sites determining Ho-2 and S hemoglobin are separate the doubly affected individuals of this kindred may be properly referred to as double heterozygotes. This term is often inappropriately applied to such conditions as hemoglobin S-C disease where in fact, as shown by Hunt (8) and by Itano (9) both abnormalities are the result of modifications of the β polypeptide chain. These abnormalities are apparently allelic. Affected individuals might best be characterized as "doubly abnormal."

Insufficient data are available to establish or reject linkage between the genetic loci controlling Ho-2 hemoglobin and any of the blood or serum groups examined. Nonetheless no example of extremely close linkage was observed. Stocks of sera from most family members have been stored at $-15°$C. with the view that the kindred may be re-examined for linkage as new stable serum polymorphisms are discovered.

In no case did blood or serum group data provide any evidence that relationships were other than as shown.

SUMMARY

The kindred originally described by Smith and Torbert has been re-examined. Their proposal that Hopkins-2 and sickle cell hemoglobin are determined by genetically distinct loci is confirmed beyond reasonable doubt. No apparent extremely close linkage exists between these loci or between the Hopkins-2 locus and any blood or serum group studied.

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CITRIC ACID CYCLE IN OSTEOBLASTS AND OSTEOCLASTS

A Histochemical Study of Normal and Parathormone-treated Rats[1]

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One of the first clues that the oxidative enzymes of the Krebs citric acid cycle might be directly involved in bone deposition and resorption was the finding of Dickens (1, 2) that the largest proportion of body citrate resides in the skeletal system. In the mouse 70 per cent of all the citrate is in the skeleton, where it is believed to be concentrated in the metaphyses (1); in the herring up to 5 per cent of the dry fat-free weight of bone is citrate (3). In 1952 Dixon and Perkins (4) reported that in bone isocitric dehydrogenase was disproportionately much less active than citrogenase and aconitase. It was tentatively suggested that citrate formed enzymatically might be co-precipitated with bone salt before it could be further oxidized, thus accounting for part of the citric acid in bone (5). Van Reen (6) recently has reported values of isocitric dehydrogenase activity in femur sections (especially those consisting mainly of marrow or epiphyseal cartilage) of young rabbits that are "many times higher" than the values reported by Dixon and Perkins (4). Both groups reported that cortical bone had little activity. In view of Van Reen's data it seems unlikely that a serious imbalance *normally* exists between rates of formation and utilization of citrate in bone as a whole. According to the recent assays conducted by Borle *et al.* (7) on normal mouse metaphyses, the accumulation of lactate by osseous tissue is far more impressive (by a factor of 70) than that of citrate. Most investigators agree, however, that whether lactate metabolism is affected or not, citrate production by bone is enhanced in hyperparathyroidism (8). Much of the evidence supporting the argument that the parathormone-induced mobilization of calcium is mediated through regulation of citrate metabolism in bone, and probably kidney, has been discussed recently by Neuman and Neuman (9). If little is known about the mechanism of calcium mobilization, still less is known about the chemistry of bone resorption.

As impressive as are the histologic alterations in bone under the influence of toxic dosages of parathormone (10), it has not been possible to closely correlate the histologic events with the chemical changes (11). By applying to studies of bone the recently developed histochemical meth-

[1] Aided by a grant from the National Foundation.

ods for assaying several different oxidative enzymes, it may be possible to obtain information essential to the integration of the morphologic with biochemical data (12, 13). The primary objective of the present investigation is to study the effect of high levels of parathormone on the histochemical reactions for different oxidative enzymes in the various cell types present in developing bone.

MATERIALS AND METHODS

Experimental Animals. Rats (obtained from Carworth Farms, Inc., Rock City, N. Y.) of both sexes, newborn to a few days old, were used. Littermates of the same sex and weight were paired off, one untreated for control purposes, the other injected intraperitoneally with parathormone.[2] To insure against escape of the injected hormone, the 30 gauge injecting needle was "threaded" obliquely through the abdominal wall.

Except when otherwise stated one injection of 15 USP parathyroid units of parathormone per animal was administered. That this is a toxic dose when administered to rats within a few days postnatally was indicated by the weight loss and increased mortality observed. The animals were sacrified at varying intervals of time (1 to 72 hours) after the injection. It was hoped that by examining the specimens at different post-injection time intervals, clues as to the origin and fate of the osteoclasts would be obtained. Except at the times of injection the animals were kept with their respective mothers.

Approximately thirty litters (six animals per litter) were used in this study.

Histologic Materials. Limbs and jaws were amputated from the ether-anaesthetized or decapitated animals. With a fresh razor blade specimens were quickly cut either lengthwise or transversely to expose the bone centers. The specimens removed from one side of the body were dropped in isopentane at −70°C., transferred to the cryostat, mounted and sectioned at 4 microns. Those from the opposite side of the body were treated with 8 per cent unneutralized formaldehyde (20% formalin) at 5° to 10°C. for from eight to sixteen minutes prior to quenching at −70°C. (14). The formalin-fixed specimens were sectioned at a thickness of 1 to 3 microns and used only for the succinic dehydrogenase assays. The application of formalin fixation in histochemical studies of the oxidative enzymes has been properly evaluated in the case of succinic dehydrogenase but not in the case of the other enzymes referred to in this study (14).

Incubation Conditions.[3] The incubation conditions of the original Nitro-BT[4, 5] methods were used in the assay for succinic dehydrogenase (15), DPNH diaphorase (16), TPNH diaphorase (17), the following DPN-linked dehydrogenases: lactic, malic, isocitric, D-β-hydroxybutyric and glutamic (16); and the following TPN-linked dehydrogenases: isocitric, glutamic, malic, glucose-6-phosphate, and 6-phosphogluconic (17). In

[2] The preparation of parathormone was generously provided by the Eli Lilly and Co., Indianapolis, Illinois, through the kindness of Mr. E. E. Kennedy.

[3] Substrates and enzymes were obtained from the Sigma Chemical Co., St. Louis, Mo.

[4] Nitro-BT was purchased from the Dajac Laboratories, Division of the Borden Chemical Co., Philadelphia 24, Pa.

[5] It is customary to employ the following abbreviations: diphosphopyridine nucleotide, DPN; triphosphopyridine nucleotide, TPN; their respective reduced forms DPNH, TPNH; and 2,2′-di-(para-nitrophenyl)-5,5′-diphenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene) ditetrazolium chloride, Nitro-BT.

addition, assay of another DPN-dependent enzyme, α-ketoglutaric dehydrogenase, was conducted in a medium containing: 0.2M α-ketoglutarate, 0.1M Sorenson's phosphate buffer at pH 7.0, 1.0 mg. per ml. of diphosphopyridine nucleotide and 0.3 mg. per ml. of Nitro-BT. In those instances where the reaction was weak or absent the period of incubation was extended to as long as 4 hours.

The only modifications of the original methods (16, 17) were the use of glutamate at a final concentration of 0.5M and the addition of 0.5 mg. of DPN and TPN per ml. of their respective incubating solutions every hour. For a description of the procedures in detail and a discussion of the rationale upon which the histochemical reactions is based, the earlier papers may be consulted (14 to 17).

RESULTS

Normal, Untreated Animals (*Figs. 2 to 8*). Table I is a compilation of the findings made in the present histochemical study of normal skeletal specimens. The different oxidative enzymes assayed are listed vertically in the left-hand column of the table; the various cellular elements examined are listed horizontally at the head of the table. By employing the symbols 0, +, 2+, and 4+ to mean negative, weak, moderately intense, and very intense staining reactions, respectively, an attempt was made to quantitate the results. A 4+ reaction was one that approached maximum coloration without over-staining in 30 minutes. After 30 minutes of incubation a 2+ reaction was about half the intensity of the 4+ reaction, etc. Some of the illustrations used are of specimens that were incubated for longer than 30 minutes, as noted in the legends.

The Osteoblast. From endosteal and periosteal osteoblasts weak reactions were elicited for the TPN-linked isocitric dehydrogenase (Fig. 3), and the DPN-linked glutamic dehydrogenase. Significantly, reactions for α-ketoglutaric and succinic dehydrogenases were not detected (Figs. 4 and 5). Moderate reactions for the DPN-linked lactic (Fig. 2) and malic (Fig. 7) dehydrogenases and DPNH diaphorases were also noted in the osteoblasts. From the foregoing histochemical results it would appear that metabolism via the Krebs cycle in osteoblasts is limited by the lack of succinic dehydrogenase activity (see Fig. 1). In the absence of both α-ketoglutaric and succinic dehydrogenase activities in the osteogenic cells, the Krebs cycle would be functional only from malate to glutamate. We must assume, however, that the conditions under which α-ketoglutaric dehydrogenase activity was assayed were decidedly suboptimal. No thorough attempt was made in the present investigation to determine the optimum conditions for the histochemical assay of this enzyme.[7]

[6] To indicate the intensity of the histochemical reactions in Figure 1 and Table I, symbols have been employed as follows: 0 = negative reaction; + = weak reaction; 2+ = moderately intense reaction; 4+ = very intense reaction.

[7] Addition of 0.3 mg. of coenzyme A (obtained from Sigma Chemical Co.) to 3 ml. of incubation solution reduces the time for this assay by several fold.

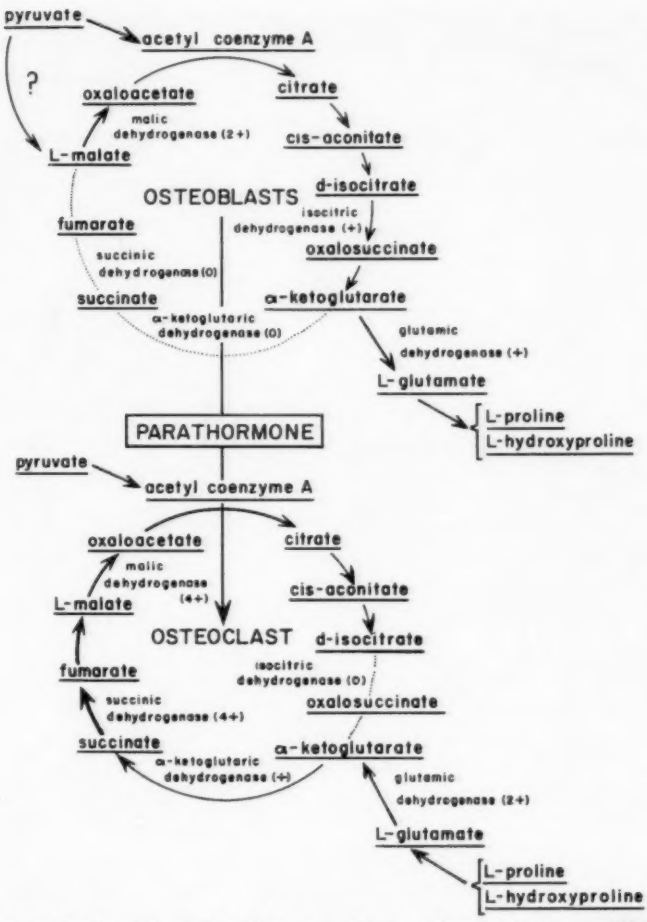
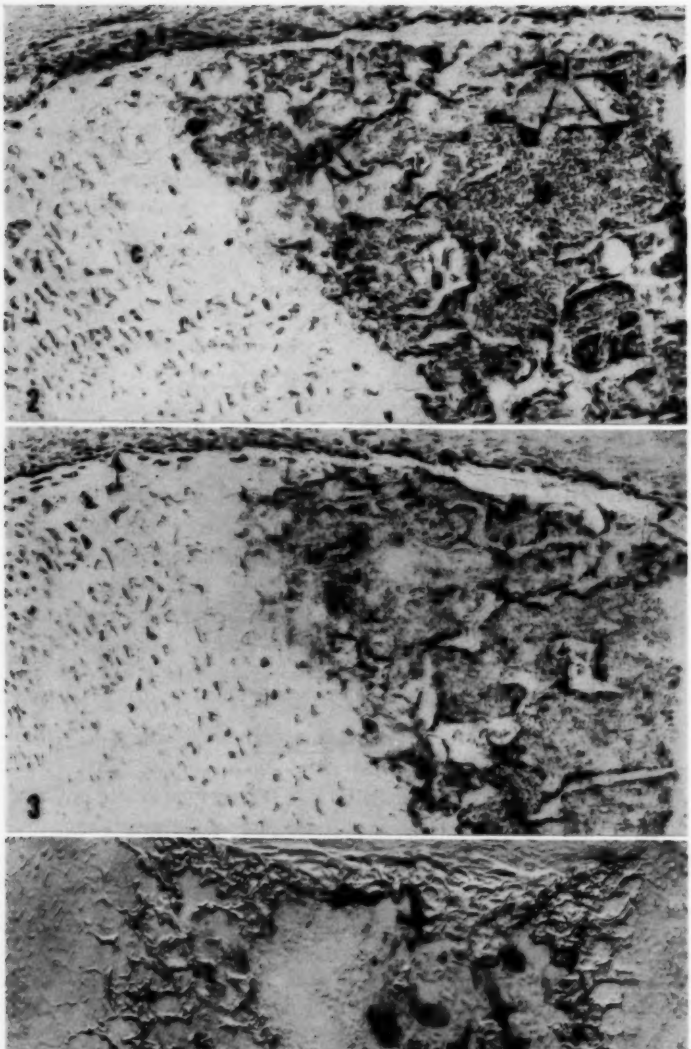


FIG. 1. The Krebs citric acid cycle in osteoblasts and osteoclast as modified on the basis of evidence gained from the histochemical assays of the several different dehydrogenases involved.[6, 8] It is proposed that the Krebs cycle in bone cells represents a target of action of parathorome. The principal influence of the hormone, as conceived here, would be the activation of the glutamate-to-oxalacetate pathway, a metabolic change associated with the conversion of osteoblasts into osteoclasts. The glutamate is believed to be derived mainly from the hydroxyproline and proline of the collagen present in the bone matrix undergoing resorption. The citrate and lactate unmetabolized in the absence of isocitric dehydrogenase activity would help to promote demineralization of the matrix.

[8] Confirmation of the data presented here, based upon study of osteoclasts in frozen sections of bone, was obtained by examination of osteoclasts in *fresh* bone marrow smears assayed cytochemically for the same oxidative enzymes.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4

The Osteocyte. The reactions elicited from the osteocyte were essentially the same as those described for osteoblasts except that in the osteocyte no DPN-linked glutamic dehydrogenase or TPN-linked isocitric dehydrogenase was demonstrable.

The Osteoclast. The multinucleated, giant cell of bone, the osteoclast, proved to be as distinctive histochemically as it is morphologically. As indicated in Table I and Figure 1, the osteoclast showed intense reactions for succinic (Fig. 4), malic (DPN-) (Figs. 7 and 8) and lactic (DPN-) (Fig. 2) dehydrogenases as well as for DPNH diaphorase, moderately intense reactions for glutamic (DPN-) and D-β-hydroxybutyric (DPN-) dehydrogenases, an inconsistent but usually weak reaction for α-ketoglutaric (DPN-) dehydrogenase and no reaction either for TPNH diaphorase or for the following TPN-linked dehydrogenases: isocitric (Fig. 3), glucose-6-phosphate and 6-phosphogluconic. These findings indicate that in the osteoclast the Krebs cycle is modified to form lactate and citrate (and presumably cisaconitate and isocitrate, too) in part at the expense of glutamate and that the pentose shunt is not operative. No suggestion can be offered here as to the possible significance of the D-β-hydroxybutyric dehydrogenase activity in osteoclasts.[8]

Epiphyseal Cartilage Cells. The reactions elicited from the cartilage cells of the epiphyseal plate, in general, resembled those demonstrable in osteoblasts, as described above (Figs. 2 to 5). However, D-β-hydroxybutyric (DPN), glucose-6-phosphate (TPN) and 6-phosphogluconic (TPN) dehy-

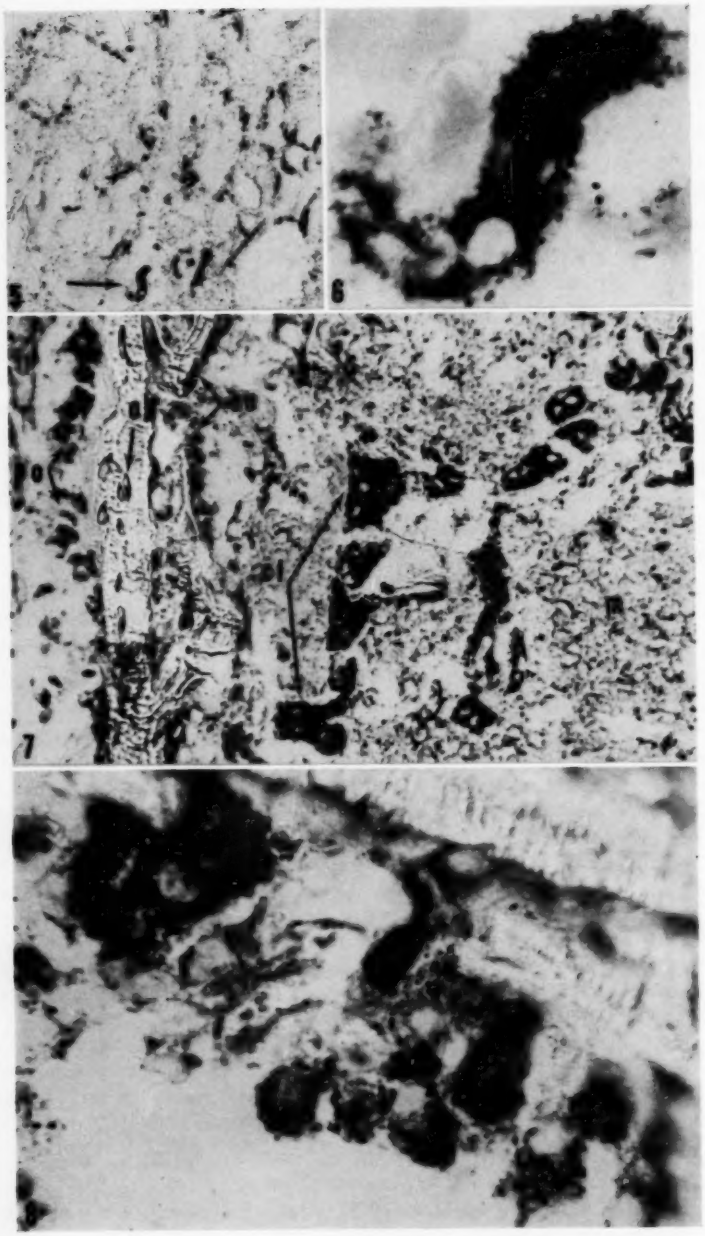
FIG. 2. Lactic dehydrogenase (DPN-) activity in a normal two-day-old rat tibia (proximal end). All cells have been stained but the reaction in osteoclasts (cl) is bolder than that of any other type of cell in the section. c = cartilage (epiphyseal plate), eo = endosteal osteoblasts, m = myeloid tissue, o = osteocyte, po = periosteal osteoblasts. Thirty minutes of incubation (×150).[9, 10]

FIG. 3. Demonstration of isocitric dehydrogenase activity required a comparatively long incubation period before osteoblasts and cartilage cells were stained. The activity in the cartilage cells of the epiphyseal plate and the endosteal osteoblasts was somewhat variable, whereas the reaction in the periosteal osteoblasts was consistent. No reaction was elicited from osteoclasts and only a weak reaction from most of the myeloid elements. This section was obtained from the same block as that illustrated in Figure 2. Four hours of incubation (×150).

FIG. 4. Succinic dehydrogenase activity in the metacarpal of a normal rat, two days of age. The osteoclasts have been selectively stained. More impressive than the difference in isocitric dehydrogenase activity of osteoclasts and osteoblasts is the difference in succinic dehydrogenase activity. Thirty minutes of incubation (×150).

[9] Photomicrographs were prepared by Mr. Chester Reather, Director of the Department of Photography at the Johns Hopkins Hospital.

[10] Figures 2 to 17 represent undecalcified, longitudinal sections, 4 to 6 microns thick, of infantile rat limb bones.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7

drogenase activities which were not elicited from osteoblasts were detected in the hypertrophic cartilage cells.

Myeloid Tissue. Because the myeloid elements are in close proximity to sites of active bone formation and resorption, it was important that they also be assessed for oxidative enzymatic activities. With the exception of the assays for DPNH diaphorase and malic and lactic dehydrogenases, the histochemical reactions for the oxidative enzymes tested were very weak or negative (Figs. 2 to 8).

Histochemical Reactions of Rats Treated with Parathormone (Figs. 9 to 17). Parathyroid extract did not alter the histochemical reactions of a given cell type as described above, despite the occurrence of the well-known dramatic shift in proportions of osteoblasts and osteoclasts. The most obvious manifestations of this shift in the metaphysis after 12 to 48 hours of hyperparathyroidism were the widespread disappearance of endosteal osteoblasts and spongy bone, and the simultaneous appearance of an increased concentration of osteoclasts.

In control specimens the endosteum consists essentially of a single layer of osteoblasts which lines all trabecular and cortical surfaces within bone. This endosteal investment is normally defective only in those restricted areas subject to remodeling. Incidentally, in such areas the presence of osteoclasts and evidence of bone resorption will usually be demonstrable. In the hyperparathyroid bones the marrow cavity was more or less free of spongy (trabecular) bone and contained an increased concentration of osteoclasts (Figs. 9 to 11) and marrow elements (including spindle cells). In the histochemical preparations only the osteoclasts are boldly stained.

FIG. 5. A portion of the metaphysis at the proximal end of the tibia. Alpha-ketoglutaric dehydrogenase (DPN-) activity was successfully demonstrated only in osteoclasts. Even if the conditions of this assay were suboptimal, since no cofactors other than DPN were added to the medium, the enzyme was active enough to permit intense staining of the osteoclast. Four hours of incubation (×150).[7]

FIG. 6. An enlargement of the osteoclast identified by an arrow in Figure 5. The osteoclast is easily recognized in normal bone on the basis of its large size and multinucleation (×1500).

FIG. 7. A portion of the diaphyseal cortex of a normal two-day-old rat stained for malic dehydrogenase. Malic (DPN-) like lactic (DPN-) dehydrogenase activity, though demonstrable in all cell types examined, was most intense in osteoclasts. cl = osteoclasts, eo = endosteal osteoblasts, m = myeloid tissue, o = osteocyte, po = periosteal osteoblasts. Thirty minutes incubation (×300).

FIG. 8. A different field from the specimen illustrated in Figure 7. That a transformation of osteoblasts into an osteoclast may be under way is indicated by the fact that osteoblasts have clustered close together (though the boundaries of each are still distinct) and simultaneously show an intensification of the enzymatic (malic dehydrogenase) activity. Adjacent to the cluster of osteoblasts is an osteoclast (×1000).

TABLE I

Results of the Histochemical Assays for Various Dehydrogenases and Diaphorases in Developing Bones of Infant Rats[6]

Enzymes assayed	Epiphyseal cartilage cells	Bone cells				Myeloid tissue
		Osteocytes	Osteoclast[5]	Osteoblasts		
				Periosteal	Endosteal	
Flavo-Protein						
Succinic Dehydrogenase	0	0	4+	0 or +	0	0 or +
DPNH Diaphorase	2+	2+	4+	2+	2+	+ or 2+
TPNH Diaphorase	0	0	0	+	+	0 or +
DPN-linked Dehydrogenase						
α-Ketoglutaric	0	0	+	0	0	0
D-β-Hydroxybutyric	2+	0 or 2+	2+	+	0	0 or +
Glutamic	+	0	2+	+	+	0 or +
Isocitric	0	0	0	0	0	0
Lactate	2+	+ or 2+	4+	2+	2+	+ or 2+
Malic	2+	+ or 2+	4+	2+	2+	+ or 2+
TPN-Dehydrogenase						
Glucose-6-phosphate	+	0	0	+	0	+ or 0
Glutamic	0	0	0	0	0	0
Isocitric	+ or 0	0	0	+ or 2+	+	0 or +
Malic	0	0	0	0	0	0
6-Phosphogluconic	+	0	0	+	0	+ or 0

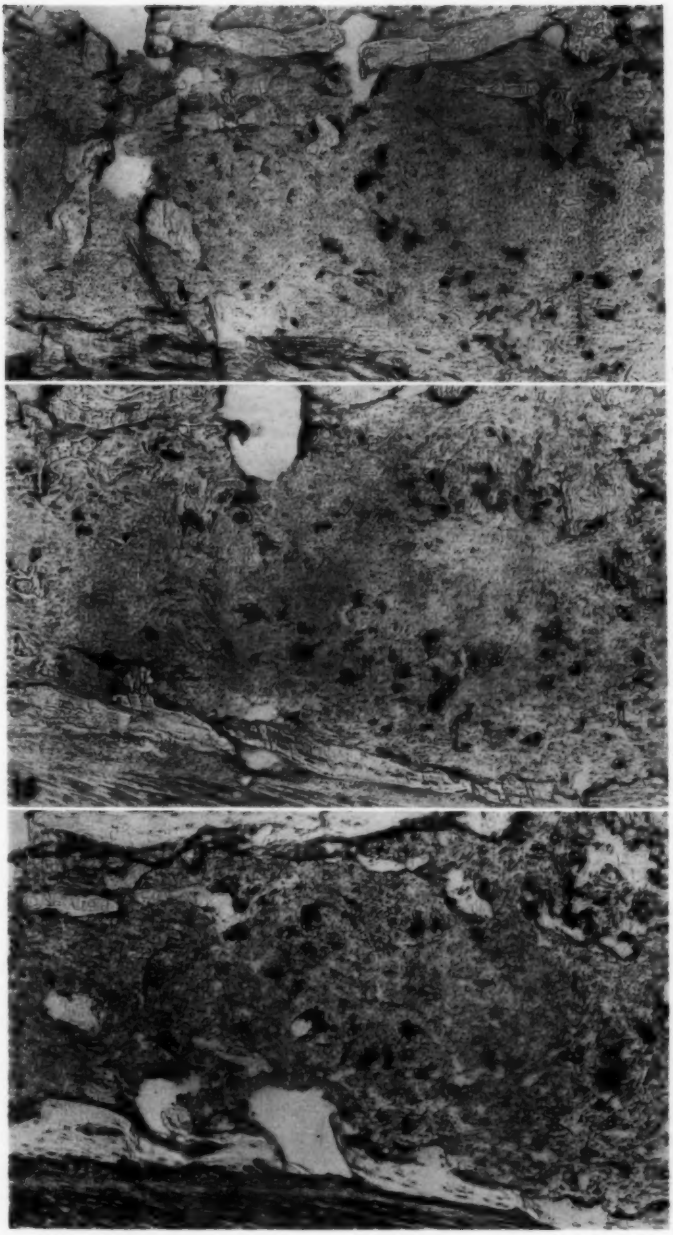
The myeloid elements, though densely arranged and occupying most of the marrow cavity are but faintly stained.

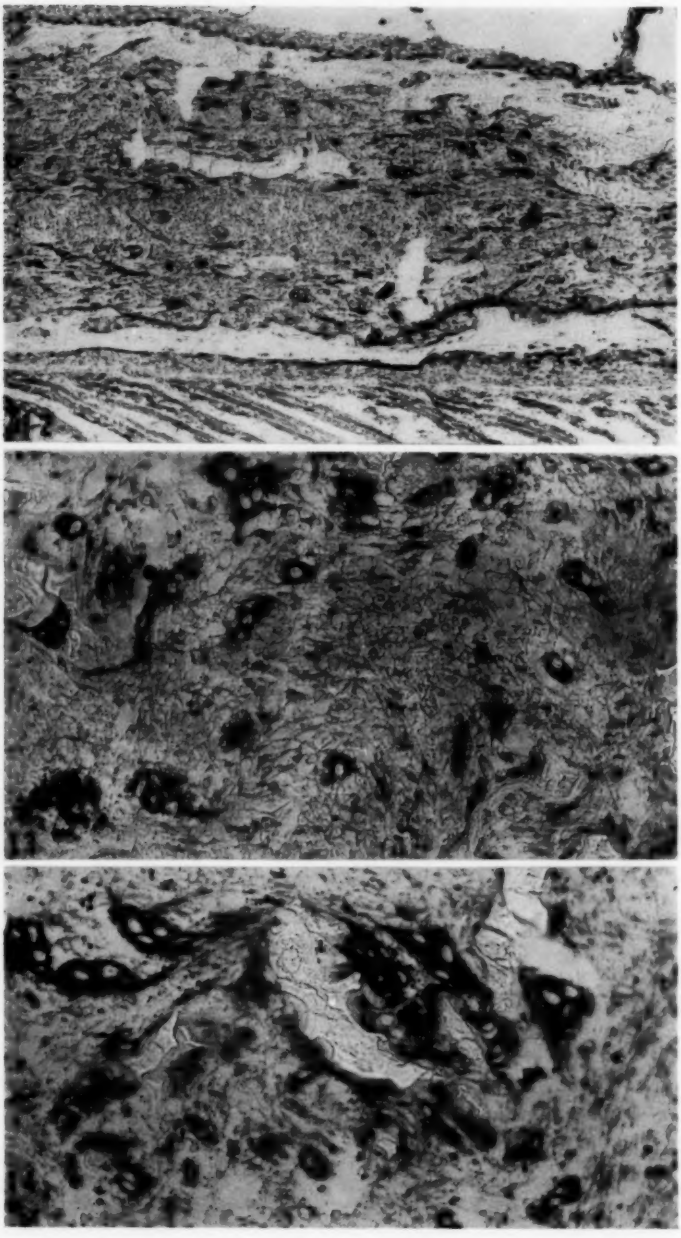
Cells whose cytoplasm showed the same histochemical reactions as the

FIG. 9. The tibia of a two-day-old rat that had received eighteen hours previously, 15 USP parathyroid units of parathyroid extract intraperitoneally. An intense reaction for succinic dehydrogenase was elicited only from the osteoclasts, a swarm of which are seen stretched across the marrow cavity in the right half of the field. Much of the trabecular bone has already been resorbed. Cortical bone can be seen near the upper margin of the photomicrograph. Sections were cut within 6 microns of those shown in Figures 10 and 11. No fixation was used (×100).

FIG. 10. The tibia of a two-day-old rat that had received, eighteen hours previously, 15 USP parathyroid units of parathyroid extract intraperitoneally. The reaction for the DPN-linked glutamic dehydrogenase closely resembles that for succinic dehydrogenase at this magnification. Sections were cut within 6 microns of those shown in Figures 9 and 11. No fixation was used (×100).

FIG. 11. The tibia of a two-day-old rat that had received eighteen hours previously, 15 USP parathyroid units of parathyroid extract intraperitoneally. The DPN-linked malic dehydrogenase was detectable in apparently all of the cell types that might be found in or near developing bone. However, the reaction elicited from the osteoclasts was always more intense than that of any other cell type in the specimen. Sections were cut within 6 microns of those shown in Figures 9 and 10. No fixation was used (×100).





osteoclast but with only one or two nuclei were encountered rather frequently in hyperparathyroid specimens (Figs. 13 and 16) but were rarely seen in normal specimens. No quantitative study was conducted to determine whether or not a correlation existed between number of nuclei per osteoclast and duration of hyperparathyroidism. However, the impression was gained that after 12 to 18 hours of hormone administration uni- and binucleated cells with osteoclast-like cytoplasmic reactions were easily demonstrable; whereas, after 36 to 48 hours of therapy the smallest elements were much less frequently seen and enormous osteoclasts with as many as 2 or 3 dozen nuclei were noted. This suggests that parathormone, when administered at toxic dosage levels, promotes syncytium formation; hence, perhaps, osteoclasts of increasing size and increasing numbers of nuclei are formed from uninuclear precursors.

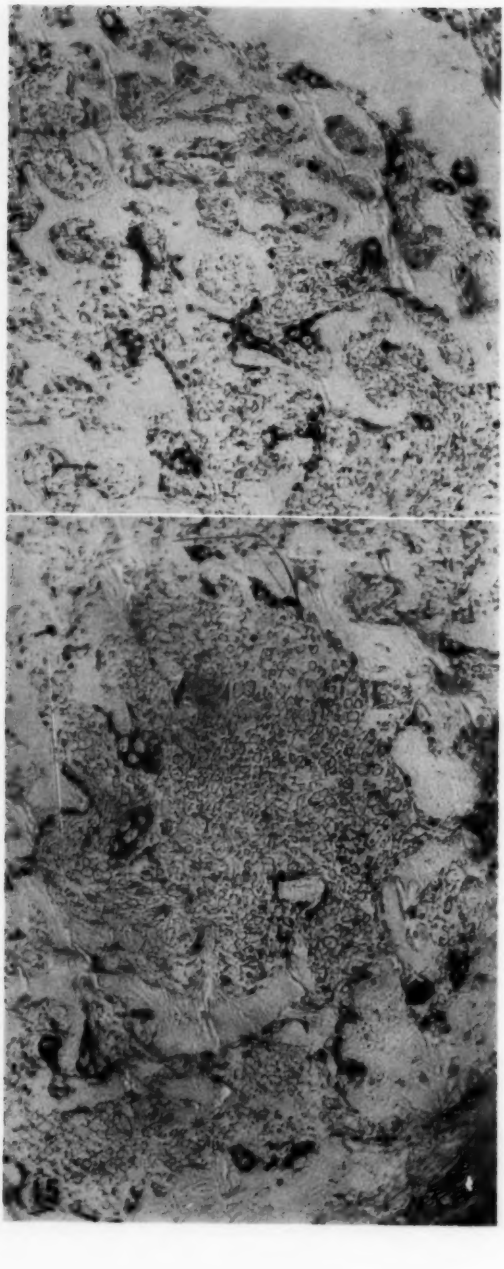
The osteoblasts of the periosteum seemed less like endosteal osteoblasts and more like fibroblasts in their response to parathyroid extract administration. The periosteal osteoblasts showed little evidence of participating in the large scale transformation to osteoclasts as manifested by the endosteum, a finding consistent with the results obtained by Tonna (18).

To sum up the effect of parathormone, as indicated by these experiments, it may be said that in shifting the proportion of osteoblasts to osteoclasts parathormone thereby effects a dramatic shift in the proportion of cells capable of forming citrate and lactate at the expense of glutamate.

FIG. 12. Parathormone treated, unfixed material. The DPN-linked malic dehydrogenase in the tibia of a three-day-old rat 24 hours after receiving 15 USP units of parathyroid extract, intraperitoneally. The periosteal osteoblasts (a single row of which are seen on the external surface of the cortical bone near the upper margin of the illustration) are stained rather intensely and show no sign of "breaking ranks" to coalesce and form osteoclasts. The internal aspect of the cortical bone lacks a protoplasmic lining; in fact, prospective endosteal osteoblasts are few and far between. The many osteoclasts seen are very intensely stained and for the most part are not in close proximity to bone matrix. In normal specimens osteoclasts are not only less numerous but almost always in close proximity to bone matrix (×100).

FIG. 13. Parathormone treated, unfixed material. Femur assayed for succinic dehydrogenase 12 hours after the administration of 15 USP units of parathormone. In this small, circumscribed area a large number of intensely stained independent elements is seen. Those that appear to be uninucleated are presumed to represent osteoclastic precursors which have responded metabolically to the influence of parathormone but have not yet coalesced with like cells. Another possibility is that the unicellular elements are multinucleated osteoclasts sectioned in such a plane as to isolate a portion containing one nucleus from the rest of the cell. Thirty minutes incubation (×300).

FIG. 14. Parathormone-treated, unfixed material. A specimen removed from the same block as that from which Figure 13 was made. By extending the incubation period to one hour, the intensity of the stain representing glutamic dehydrogenase activity in osteoclasts is equivalent to that obtained after one-half hour in the succinate medium (×300).

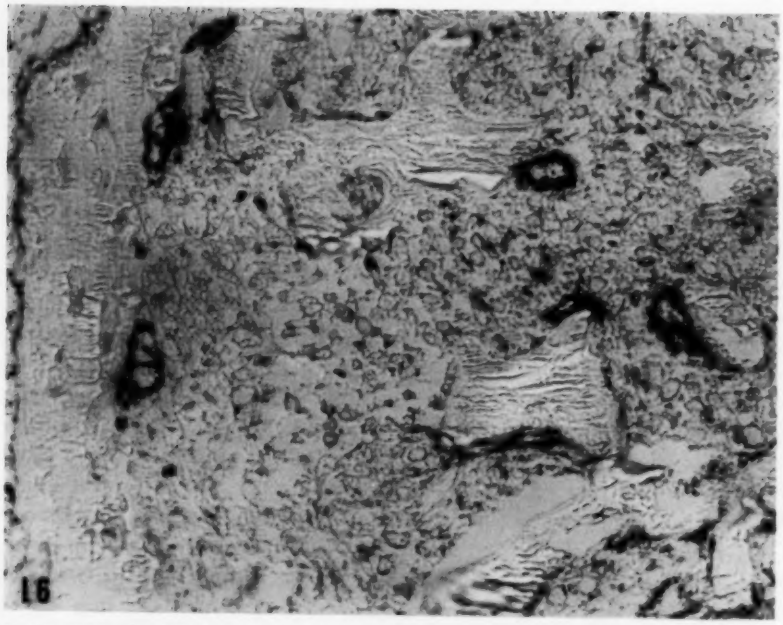


DISCUSSION

The results of the histochemical assays for isocitric dehydrogenase in developing bone are reasonably consistent with the recent biochemical assays conducted by Van Reen (6). In the present study it was found that osteoblasts and epiphyseal cartilage cells showed weak isocitric dehydrogenase activity whereas the osteocytes and osteoclasts lacked this enzymatic activity. Since cartilage cells (19), osteoblasts and even osteocytes have rich cytoplasmic glycogen stores (8, 19, 20), it is assumed that lactate (7) and citrate are produced by these cells at the expense of carbohydrate. In acute hyperparathyroidism there is an increased rate of citrate (8, 21) and lactate (22) accumulation, yet at the same time metaphyseal osteoblasts and osteocytes diminish in number (10). Since osteoclasts are poor in glycogen, one might suspect that the continued high rate of citrate and lactate production in hyperparathyroidism, attended by increased numbers of osteoclasts, requires a supply of substrate in excess of that provided by the blood stream or derived from the glycogen that existed in osteoblasts before undergoing transformation into osteoclasts. Others (8, 22) have reached a similar conclusion. Present histochemical findings lead one to suspect that additional substrates may be obtained from the matrix. Collagen is singularly rich in hydroxyproline and proline, amino acids that can be converted to glutamate.

If the initial parathormone-induced metabolic change in bone cells is the complete suppression of isocitric dehydrogenase activity, as an immediate consequence there would be the already known increase in citrate accumulation. Decalcification of the matrix presumably, therefore, would precede degradation of the organic components of bone, a belief that is held by others (9). Wherever the apatite crystals have been removed, collagen would be exposed and subject to degradation. The enzymes necessary to

FIG. 15. An oblique section through the metaphysis of the tibia of a two-day-old rat that had received 15 USP units of parathyroid extract 12 hours prior to autopsy. The block was exposed to 20% formalin for 12 minutes at 5°C. before frozen sections were prepared. The reaction for succinic dehydrogenase is shown after an incubation period of forty minutes. The marrow spaces are replete with small cells. A substantial meshwork of trabecular bone is still present. The cytoplasm of the osteoclast alone has been intensely stained. Much of the trabecular bony surface is covered by thin films of intensely stained cytoplasm. Where these films are in direct continuity with the main nucleated portion of the cell the identity is obvious; but where the plane is such as to reveal the film unconnected to the main body of the cell one is not certain that the latter also represents osteoclastic protoplasm (×250).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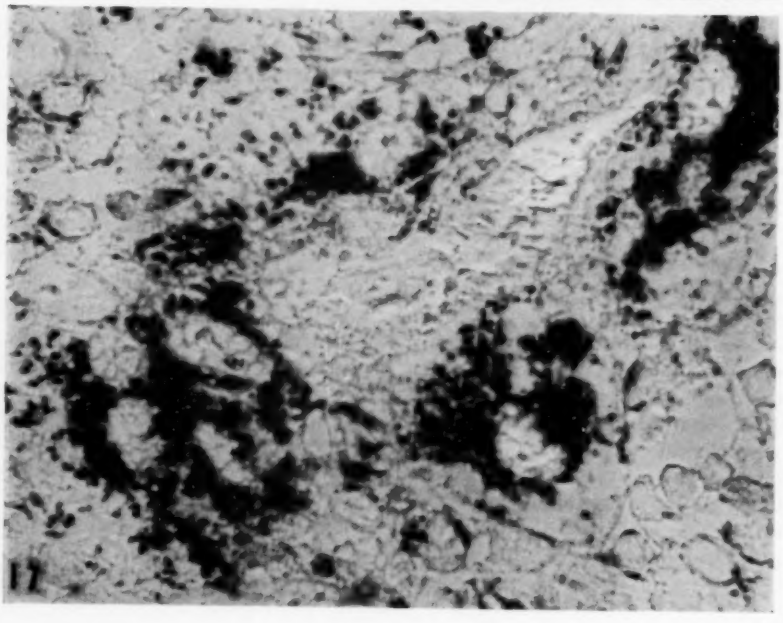
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oxidize glutamate to oxalacetate seem to be particularly active in the osteoclast. From oxalacetate it is possible to obtain:

1) *citrate* by the condensing reaction,

acetyl-coenzyme A + oxalacetate = citrate, or

2) *lactate* by a two-step reaction, the first of which, catalyzed by oxalacetate corcarboxylase, converts oxalacetate to pyruvate; the second, catalyzed by lactic dehydrogenase in the presence of DPNH, converts pyruvate to lactate. An intense reaction for lactic dehydrogenase was demonstrated in the osteoclast. It is reasonable to assume that acetyl-coenzyme A and cocarboxylase are also present. The newly-formed citrate and lactate would solubilize more of the matrix-bound calcium to perpetuate the process of bone resorption. By a rather short sequence of enzymatic activities two important derivatives of bone matrix (glutamate and calcium) are eliminated from the site.

The parathormone-promoted modification of the Krebs citric acid cycle in bone cells provides insight into the mechanism by which a distinctive cell type may be morphologically and physiologically transformed, perhaps as an adaptive response to a metabolic requirement of bone absorption. This process may be contrasted with the "cellular modulation" of Bloom (23), "... in which, despite marked changes in the morphology of cells, there is no loss or gain in cellular potentialities." The morphological changes observed in the histochemical preparations are gratifyingly consistent with those of the classical work of Heller, McLean and Bloom (10), who observed that without mitoses, migration or death, the cells concerned with formation and maintenance of bone matrix within a few hours may become engaged in matrix resorption. Tonna and Pillsbury (24) from their studies of Regaud preparations (in which mitochondria are selectively stained) conclude that osteoclasts may arise through a fusion of osteoblasts, a transformation accompanied by a manifold increase in mitochondrial concentration. Counterstains were not used in the present histochemical study so that observations were limited to the cells manifesting activity of the enzymes assayed. In specimens assayed for the ubiquitous DPNH diaphorase cartilage cells, osteoblasts, osteocytes and osteoclasts were all well

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FIG. 16. Succinic dehydrogenase in parathyroid-treated formalin-fixed material. Although possessing only one or two nuclei, the darkly stained cells are believed to be osteoclasts because of their intense reaction for succinic dehydrogenase, a finding that helps to differentiate -clasts from all other cell types found in developing bone (×500).

FIG. 17. Succinic dehydrogenase in parathyroid-treated formalin-fixed material. Four osteoclasts deployed about one end of a trabecula of bone are shown stained for succinic dehydrogenase. The formazan is confined to discrete spheres or short rods which are highly concentrated in all parts of the cytoplasm and are believed to represent the mitochondria (27) (×1400).

stained and as readily identifiable as in specimens prepared by classical histologic methods. However, in the histochemical preparations identification of the spindle cells, though carefully described by Heller, *et al.* (10), was not accomplished due to their very low level of oxidative enzymatic activity and also because of the fact that the spindle cells are known to be intermingled with the heterogeneous, poorly differentiated myeloid elements (10). The spindle cells apparently have a poorly developed chromidial substance and Golgi apparatus as well as a low level of oxidative enzyme activity. They are not immediately concerned with bone matrix formation or destruction, but presumably represent resting, depressed or involuted bone cells.

The possibility that phagocytosis (as classically conceived) plays a role in resorption of bone matrix was ruled out decisively (10, 25). The possibility that proteolytic enzymatic action may play an important role in the resorption process has been frequently mentioned and recently osteoclasts have been shown to exhibit "high" acid phosphatase (12, 26, 27), esterase (28), and aminopeptidase (12) activities. It is only reasonable to suppose that a variety of hydrolytic enzymes would play an important role in all but the final sequence of this complex catabolic process. However, as yet too few data are available to attempt to discuss the role of the hydrolytic enzymes in bone resorption.

The suggestion has been made repeatedly that osteoclasts represent the terminal stage of dying cells which even before coalescence have been under duress (low oxygen tension, etc.) and "leaking proteolytic and other enzymes" (9). The observations of the present and other recent investigations (12, 13, 24 to 30) would certainly argue against the latter speculation. If anything, the osteoclasts represent most intensely active elements. Morphologically the osteoclast has an exceptionally high concentration of mitochondria (24) (see Fig. 17). Goldhaber (29) found that hyperoxia causes an increased rate of resorption of bone matrix in cultures of calvariae. By employing time-lapse micro-cinematography he found that osteoclasts showed "active mobility" and "participated directly in the process of bone resorption." Goldhaber suggests that, "the effect of hypoxia may be similar to that of parathyroid hormone, both agents modifying the metabolic pathway of bone resorbing cells or their precursors and resulting in the local accumulation of substances required for bone resorption." Using the same type of preparation, it was noted subsequently that, paralleling the resorption of bone morphologically, increases in citric acid (. . . 8–19) times greater than the total content of the original calvaria . . .), calcium and phosphorus were observed" (30). Judging from the intensity of the histochemical reactions for oxidative enzymes and the concentration of the mitochondria (24), one must assume that the osteoclast has a very high rate of oxygen utilization and would not be expected to

function optimally in an environment of low oxygen tension. In the tissue culture, apparently if the osteoclasts are to thrive, a high level of oxygen tension must be maintained.

The present investigation offers no direct observations that reveal the fate of the osteoclast. It has been claimed that the osteoclast is an ephemeral element whose life span is less than 48 hours (9, 31, 32). According to the histochemical assays the osteoclast is a highly specialized cell which, since it lacks isocitric dehydrogenase activity, must rely heavily on glutamate as a source of energy. As is so often the case, histochemical findings are subject to certain criticisms. Perhaps under the conditions of the histochemical assay, isocitric dehydrogenase is either not active or tends to escape from the osteoclasts into the incubation solution. These possibilities seem unlikely in view of the fact when assayed by the same method a very intense reaction for isocitric dehydrogenase activity is demonstrable in the liver, kidney and intestinal epithelium. The fact that TPNH-diaphorase activity was not demonstrable in the osteoclast indicates further that a means of oxidizing citrate (or isocitrate) is not present in this cell. If it is true that the osteoclast is critically dependent upon the substrate, glutamate, one would like to know to what extent oxalacetate cocarboxylase and the transaminases participate in this cell's metabolism.

The hypothesis developed here represents an attempt to integrate some of the biochemical, histochemical and morphological data obtained from studies of developing bone. The hypothesis invites confirmation, especially by, a) quantitative assays for oxidative enzymatic activity carried out directly on pure cultures of osteoclasts, and b) electron microscopic examination of endosteal preparations likely to contain osteoblasts in the process of transforming into osteoclasts. Experimental work along these two main lines has been initiated.

SUMMARY

Bones of normal and parathormone-treated one to three-day-old rats were assayed histochemically by the Nitro-BT methods for the DPNH- and TPNH-diaphorases and for several different dehydrogenases.

From the osteoclast very intense reactions were elicited for succinic dehydrogenase and for the DPN-linked glutamic, malic and beta-hydroxybutyric dehydrogenases. Even alpha-ketoglutaric dehydrogenase activity which was not detectable in osteoblasts, osteocytes and cartilage cells was demonstrable in the osteoclasts. On the other hand, no isocitric dehydrogenase activity was detected in this multinucleated giant cell. It is proposed that the osteoclast, by maintaining a high rate of citrate and lactate producion at the expense of glutamate, actively promotes decalcification of the matrix of bone and calcified cartilage.

Osteoblasts, osteocytes and chondrocytes were found to lack succinic dehydrogenase activity but manifested at least weak reactions for isocitric and glutamic dehydrogenases.

Attention is called to the histochemical evidence supporting the hypothesis that parathormone, at least when administered in high dosages, profoundly modifies the Krebs citric acid cycle in osteoblasts and coincidently promotes the conversion of osteoblasts into osteoclasts.

ACKNOWLEDGMENT

The criticisms and suggestions of Drs. A. L. Lehninger and F. Vasington of the Department of Physiological Chemistry, of Dr. R. A. Robinson of the Department of Orthopedic Surgery, and Drs. D. Bodian, W. L. Straus, Jr., R. Bergman, and W. Young of the Department of Anatomy, The Johns Hopkins University School of Medicine, are gratefully acknowledged.

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HYPOXIA IN INFANTS AND CHILDREN: A CLINICAL-PATHOLOGICAL STUDY CONCERNING THE PRIMARY VISUAL PATHWAYS[1]

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In the absence of defective development of the eyes and of disease in the mother during pregnancy, defective vision or blindness in infants and young children usually may be attributed to damage to the cerebral visual pathways. Determination of the etiology of such damage is surrounded with difficulties. Examination findings are purely objective. Exact information concerning the infant before, at and after birth often is difficult to obtain and, if obtained, is assessed with uncertainty. Thus evaluation of the visual status in large part is based on coexisting neurologic and mental abnormalities. Hypoxia is a factor in many instances. Correlation between clinical and neuropathologic studies should increase our knowledge concerning the patterns of cerebral changes associated with hypoxia. It might be anticipated that animal studies would provide answers to the problem and such studies are vitally important but leave much to be desired. Obviously it is impossible to have the controls in humans that can be established in animal studies.

In this paper we present pathological and clinical studies concerning cerebral changes which arise in association with generalized also with localized hypoxia, granting freely that often it is impossible to know that hypoxia alone has been responsible for the changes described. The studies have suggested concepts concerning the interrelationships of the cerebral changes, and these concepts are stated herein. Because our principal interest has concerned the cerebral visual pathways it is hoped these studies may in some degree improve our understanding of some of the visual defects which occur in infants and children and may persist during life. As particularly important we have included studies on birth injuries resulting from mechanical forces acting on the fetal head because such deformities often provide focal areas of hypoxia, and secondarily may affect remote centers of vital function.

The paper is divided into three sections. Section I deals with various types of damage occurring at and immediately after birth. Section II concerns lesions originating during infancy or early childhood. Section

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III considers optic atrophy and hypoxia as a diagnostic problem in infancy and early childhood.

I. INVOLVEMENT OF THE CEREBRAL VISUAL PATHWAYS AT AND IMMEDIATELY AFTER BIRTH

Either neonatal asphyxia or birth injury is thought to be etiologic in a majority of instances of cerebral damage occurring during or immediately after birth. A minority of such cases of brain damage is attributed to placenta praevia, toxemia of pregnancy or other disease in the mother which could influence the fetus. Our studies concerning brain damage occurring at or right after birth and a relevant clinical study are now presented.

The term "asphyxia neonatorum", or "apnea neonatorum", implies absence of breathing and means that the fetus has not started breathing, not that it has started breathing and has been smothered (1). In neonatal asphyxia there is depression of the breathing center which may be explained by (1) severe hypoxia prior to birth, (2) intracranial lesions directly or indirectly involving the respiratory centers, or (3) medullary depression due to such drugs as morphine. Neonatal hypoglycemia may aggravate the condition or itself be responsible for apnea, cyanosis and convulsions (2).

It is well known and generally agreed that lack of oxygen is responsible for the greatest number of fetal and neonatal deaths. Haller, Nesbitt and Anderson (3) classified the cause of death in 1,043 infants of over 1,000 grams birth weight in the Johns Hopkins Hospital in a 12-year period, 1937 to 1949. They attributed 249 fetal and 102 neonatal deaths to anoxia —33.7 per cent of total deaths. Birth injury accounted for 9.8 per cent, and malformations for 9.4 per cent of total deaths. Prematurity was an important consideration in their series of cases. Valuable information on this and the entire subject of hypoxia is available in Ford's textbook (4). *Neurological and Psychological Deficits of Asphyxia Neonatorum* edited by Windle (1) is particularly useful.

As regards the significance of asphyxia neonatorum there is lack of general agreement. Ford (4) included mention of some observers who believe it is frequently productive of permanent cerebral damage and of others who conclude it is essentially a prolonged physiological phenomenon and of no significance. He remarked "At present it is impossible to say how frequently the baby's brain is injured at birth by anoxia and what part this condition plays in the genesis of infantile cerebral palsy".

Through the cooperation of Dr. Nicholas Eastman and Dr. Janet Hardy we obtained a list of 114 children who had failed to breathe at birth for 2 minutes or more. We were able to locate and examine 52 of these children.

The eyes were examined for external defects; visual acuity was recorded; the optic fundi were examined; confrontation visual fields were obtained. Hearing was tested and audiometric recordings were made. Routine physical and neurological examinations were made only when indications for such studies obviously were present. All children were 5 to 8 years old when examined. Our findings as regards the eyes is shown in Tables I to V below.

TABLE I

Survey Cases

Survey group	Number of cases	With ocular involvement	Percentage
Hypoxic	52	5	9.6%
Control	62	7	11.3%
Total	114	12	

TABLE II

Types of Ocular Defects Seen in Children Hypoxic at Birth

Ocular defect	Hypoxic	Control
1. Extra-ocular muscle pareses	0	0
2. Heterotropia	2	1
3. Nystagmus	1	1
4. Optic atrophy and pallor	1	0
5. Gross visual field defects	0	0
6. Cataract	0	0
7. Retinopathy	0	0
8. High refractive error	3	6
9. Unexplained visual loss	0	0
Total	7	8

TABLE III

Obstetrical and Maternal Complications Thought to be Responsible for the Hypoxia

1. Antepartum bleeding	2
2. Toxemia of pregnancy	5
3. Placenta praevia	0
4. Prolapsed cord	3
5. Premature rupture of membranes	2
6. Abnormal lie	6
7. Prolonged second stage	8
8. Tight loop of cord around neck	1
9. Diabetes	2
10. Anesthesia	1
11. Unexplained*	22
Total	52

* Breathing time 2 minutes or over.

TABLE IV

*Breathing Time**

1. Under 2 minutes	2
2. 2 to 10 minutes	47
3. 11 to 20 minutes	3
4. 21 to 30 minutes	0
Total	52

* Efforts were made routinely to initiate respirations and supply oxygen.

TABLE V

Relation of Duration of Apnea to Incidence of Ocular Defects

Ocular defect	Breathing time			
	Under 2 min.	2–10 min.	11–20 min.	21–30 min.
1. Extraocular muscle pareses				
2. Heterotropia	1	1		
3. Nystagmus		1		
4. Optic atrophy and pallor		1		
5. Gross visual field defects				
6. Cataract				
7. Retinopathy				
8. High refractive error	1	2		
9. Unexplained visual loss				
Total	2	5	0	0

Additional Data Re Tables I to V*

Two children with breathing time less than two minutes were included in the apneic group. H.W. 6616628, had a preeclamptic mother and there was fetal distress during delivery (heart rate dropped to 60 per minute). Breathing time was 1 minute. (P.L. 600143); mother had severe vaginal bleeding for 3 days prior to delivery; breathing time was immediate. Both these children were normal when we saw them.

With breathing time over 10 minutes there were three children studied (R.H. 530685); A.S. 194294) (L.B. 359319). All three had normal eyes and normal auditory acuity when examined.

One child weighed 4,260 grams at birth and had a breathing time of 4 minutes. The mother who weighed 400 pounds was diabetic. The child's eyes were normal. He was mentally retarded and all reflexes were hyperactive (G.P. 390823).

In the control group (K.G. 573338) two months premature was not apneic at birth. The mother had antepartum bleeding. The child exhibited rotatory nystagmus and had compound myopic astigmatism.

* Dr. Timoteo Jiminez examined these children and prepared the tables. We appreciate his help.

HEARING TESTS

The children studied as regards their eyes were tested for hearing status through the cooperation of Dr. John E. Bordley and Dr. Glenn Jones. It seems probable that what applies to the cerebral visual pathways as

regards hypoxia is also true as regards the auditory pathways. Dr. Glenn Jones summarized his findings as follows.

"All subjects were examined by an otologist before audiometric testing and all physical findings were negative. An audiometric screening test was given to 47 of the 52 children in the apneic group and to their paired controls. Seven tones spaced at one-octave intervals from 125 cycles per second to 8,000 cycles per second were presented to each ear by air conduction at a 15 decibel level of intensity above audiometric zero. Lack of response to more than one of the tones in either ear was considered failure. Complete threshold audiometric tests utilizing both air and bone conduction were given to those subjects failing to pass the screening test.

"In the apneic group 46 of the 47 children tested passed the screening test. One child with normal eyes (R.T. 433595) displayed a mild bilateral conductive loss with no sensory-neural component. He was recovering from a severe cold. Subsequent testing one month later gave essentially normal thresholds. G.P. (390823) was not tested. He was mentally retarded, hyperactive, and uncontrollable in the test situation. He responded overtly to sound but had gross retardation in the language and speech areas. Four children (W.H. 612415; V.W. 670022; D.Y. 449010; C.D. 591339) did not keep appointments for hearing tests and were not tested.

"There was a single failure in the control group, (V.F. 564665). Threshold audiometry disclosed a mild high-tone sensory-neural loss bilaterally, at 4,000 c.p.s. (30 decibels) and at 8,000 c.p.s. (25 decibels). This child was premature. Her mother wears a hearing aid but was not available for audiometric testing.

"Data from the hearing tests were not statistically analyzed because of the complete absence of positive sensory-neural findings in the apneic group."

Because it points up particular problems as regards evaluation of asphyxia neonatorum a case report on a child not included in the apnea neonatorum series is now summarized.

Case 1. E.R.C. (B 24736) weighed 1,975 grams at birth. Pregnancy was uncomplicated. Membranes ruptured 49 hours before delivery. Mother was given penicillin 600,000 units and streptomycin 1 gram, 8 and 4 hours prior to delivery, also morphine 16 mg. 4 hours prior to delivery. Fetal heart rate 120, 30 minutes before delivery, slowed to 20 just before delivery. Low forceps were used. There was one true knot in the umbilical cord which encircled the neck twice. Immediately after birth baby took one gasp; pale and cyanotic to a mild degree. Baby was placed in Bloxom apparatus (artificial breather) for 10 minutes, and during this time took a few gasps. Removed from the apparatus and mucus was sucked out. The infant remained pale and slightly cyanotic. After 45 minutes respirations became more rapid, having increased from 2 to 3 to 20 per minute. After a further half-hour breathing was 60 to 80 per minute, spontaneous, with expiratory

difficulty but good exchange. Heart beat was not heard for 15 minutes after birth. On the day after birth the infant was pink, crying well, limp, markedly tachypneic, twitching but not convulsing. Later in the day there developed opisthotonos and nuchal rigidity with arms and legs outstretched. On the second day after birth slight improvement was noted. It was thought there was severe brain damage. The cry remained cephalic. Thereafter there was at first slight gradual improvement and by the end of a week the improvement was pronounced. Seventeen days after birth the child was discharged from the hospital. Examinations at that time failed to reveal any abnormality. Our examinations were made when the child was 5 years of age. She seemed entirely normal. Visual acuity and hearing were normal. She was unusually attractive and bright.

How can infants such as that whose case is summarized above (Case 1, E.R.C) and those with prolonged apnea (Table IV, no. 3) fail to develop permanent damage to the brain when it is generally well known that under some circumstances apnea at birth has serious prognostic import?

Our studies on apnea neonatorum, also Case 1, suggests that in the group of children we examined there was absence of extensive intracranial involvement such as may occur with birth injury. Some degree of hypoxia exists during labor as evidenced by the cyanosis of the newborn infant. Neonatal apnea seemingly only prolongs the hypoxia which has been present prior to birth.

We have evidence to support the concept that a gradual transition into a state of severe oxygen want is less dangerous to the organism than is a rapid failure of oxygen supply. In animal experiments and in human cases Lindenberg (5a,b,d) demonstrated that with a sudden transition from normal oxygen supply to anoxia, the nerve cells undergo rapidly progressing necrobiotic changes destroying their structure within hours. Functional recovery after the onset of anoxia may occur only if the anoxia persists for just a few minutes. This is in agreement with clinical observations concerning cardiac arrest (5e). However, if the anoxia is preceded by severe hypoxia lasting for at least one hour both in experimental animals and in humans, the nerve cells do not show visible morphologic changes for a period of 12 hours or more. Because of this difference in behavior of nerve cells to anoxia of sudden onset and to anoxia which has been preceded by hypoxia, it seems apparent that the newborn infant is physiologically equipped to withstand apnea and that other factors are present when cerebral damage occurs. Such observations as have been mentioned confirm what Smith (6) considered as logical. He remarked that the oxygen saturation of arterial blood of the fetus *in utero* shows levels of approximately 50 per cent saturation; that shortly after birth the arterial oxygen saturation rises to levels of approximately 90 per cent; and that apnea neonatorum, occurring before the arterial oxygen has reached the higher extrauterine levels, should theoretically cause less brain damage since the tissues have been acclimatized to the lower fetal oxygen tension.

It seems entirely reasonable that when apnea in the newborn exceeds some time limits (which our studies do not indicate), death occurs, or brain damage persists. Only a few human cases where this has happened have been described. Scholz and Boellaard (7) described predominant nerve cell loss in the pallidum, and corpus Luysi bilaterally, occasionally in the cornu ammonis, thalamus and nuclei in the brain stem, especially the vestibular nuclei. The optic tracts in some instances show gliosis but no loss in myelinated fibers. Other portions of the primary visual pathways were not involved. According to these observers the loss in nerve cells in the pattern stated above is probably controlled by the stage of development of the brain in infancy when maturation of the myelin sheaths is most advanced in the phylogenetically old portions of the brain. It is believed that the relative maturity of these structures renders them more vulnerable to hypoxemia than the still immature cerebral cortex and striate bodies.

Ranck and Windle (8) described lesions in monkeys comparable to those described by Scholz and Boellaard (17) in humans. In the animals asphyxia neonatorum was induced in the fetuses just before term. The placenta was detached under local anesthetic keeping the membranes intact. From 11 to 16 minutes later the fetuses were delivered and resuscitated by pulmonary insufflation with oxygen. The brains showed a common pattern of structural alterations. Nuclei were symmetrically affected, particularly the nuclei of the inferior colliculi, gracile and medial cuneate nuclei, roof nuclei of the cerebellum, ventral and posterior group thalamic nuclei, globus pallidus, putamen and vestibular nuclei. They observed extensive cerebral cortical involvement in only one monkey, and remarked that there seemed to be no relation of the lesions to the vascular distribution. Windle, Becker and Weil (9) in a similar study done earlier on guinea pigs delivered after the uterine vessels or umbilical cord had been clamped, found that cerebral edema occurred only during the acute stage, that cerebral hemorrhages did not occur in animals killed by asphyxiation and in those which survived for as long as two hours, but in those which survived for more than three hours, cerebral diapedetic hemorrhages were present in all. As regards cytologic changes in the brain there were none in animals surviving less than one and a half hours, or those which survived for more than three weeks. The diversity of effects was noteworthy. They remarked on the lateral geniculate body being involved in some cases, the lateral thalamic nuclei in others. They concluded that it is impossible to state that asphyxia of short duration will not lead to nervous system changes, or that prolonged asphyxia must provide such changes.

Scholz and Boellaard (7) essentially disregarded the edema and swelling of the brain which results from acute hypoxia or anoxia. Such swelling

slows down capillary circulation by venous compression which again enhances the swelling process as has been usefully diagrammed by Argent and Cope (10) (see Fig. 1). To this diagram it might be added that pressure on veins and also on arteries develops and thus there is inadequate circulation with capillary stasis and cellular necrosis particularly in the respective arterial supply areas. Also the diagram does not reflect the results of oxygen deficiency on the heart. As the cardiac output decreases there is further development of brain edema and slowing of capillary circulation. Lindenberg (5e) has pointed out that if there is complete temporary heart failure, the necrotizing capillary stasis occurs first within the border zones of the large brain arteries (see section on temporary heart failure), and that arterial and venous obstruction lead to focal necrosis. Compression of the anterior choroidal arteries during the phase of brain edema has contributed to the necrosis in the pallida, optic tracts, corpora Luysi. Also vascular compression has contributed to nerve cell loss in Ammon's horns and the thalami (5c). This viewpoint alters the concept of selective vulnerability in some degree; it is recognized that such vulnerability exists. It is again remarked that swelling of the brain occurs with sudden development of oxygen deficiency.

The distribution of lesions mentioned above results from kernicterus and in posticteric encephalopathy. With kernicterus, neonatal apnea often is absent but attacks of respiratory failure invariably develop when the icterus becomes intensified, and the resultant hypoxia probably is a factor in the development of the lesions (11). We have observed nystagmus and abnormalities of ocular movement, also pallor of the optic discs in children who survived. Intermittent strabismus, conjugate deviations of the eyes

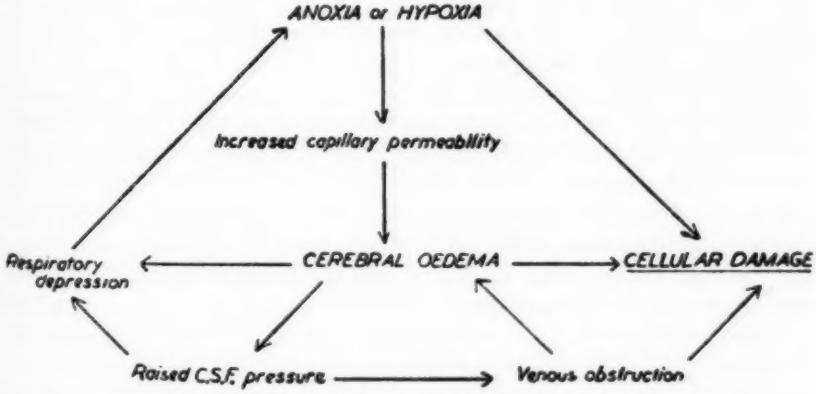


FIG. 1. (Argent and Cope). Vicious circle of cerebral hypoxia.

and demyelinization of the optic nerves found at autopsy have been observed (12).

SUMMARY REGARDING APNEA NEONATORUM

What may be termed "uncomplicated apnea" is not dangerous to the fetal brain in most instances. The evidence available on human cases is small in amount; it points to predominant involvement of the brain stem and subthalamic nuclei so that motor signs and choreo-athetosis develop. The primary visual pathways probably are seldom affected but in some instances the optic tracts are endangered. Kernicterus cannot be considered as associated with uncomplicated apnea, but with it the lesions which have been found rarely to result from apnea are essentially characteristic (7).

ASPHYXIA DUE TO CEREBRAL COMPLICATIONS

Delay in the onset of respirations or respiratory distress developing after normal breathing has been commenced may be due to depression of the respiratory centers as a result of distant lesions originating as complications of the birth process. Such complications are now considered.

BIRTH INJURY

The term requires clarification because by some observers it includes all types of brain damage occurring at the time of birth. The term should be reserved for lesions related to mechanical stresses acting on the brain or its surrounding structures during the passing of the head through the birth canal. Such lesions may be divided into two groups, direct and indirect. The direct lesions constitute frank hemorrhages into the dura, subdural space, subarachnoid space and into the brain substance. Such hemorrhages originate in tears in vessels, usually veins. Also bleeding originates in seepage of blood through the walls of veins which are congested because of obstruction to venous outflow from local pressure. Such hemorrhage is "indirect" and provides for ischemic or hemorrhagic softenings or incomplete tissue necrosis resulting from compression of dural sinuses (especially the superior longitudinal sinus), of cerebral veins, and occasionally of arteries. In certain areas traumatic (primary) hemorrhages develop, and in other areas hemorrhages arise from severe congestion (secondary). The diagram of Haller, Nesbitt and Anderson (Fig. 2) has real value and is self-explanatory.

Involvement of the visual pathways as a result of hemorrhage at birth is exemplified by case studies in which autopsy findings are available. Insofar as seems reasonable the probable sequence of events is stated.

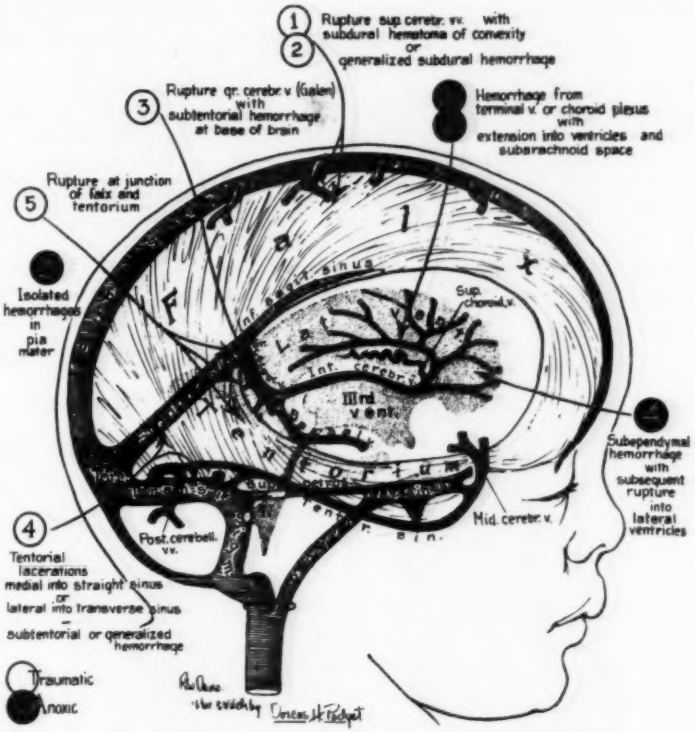
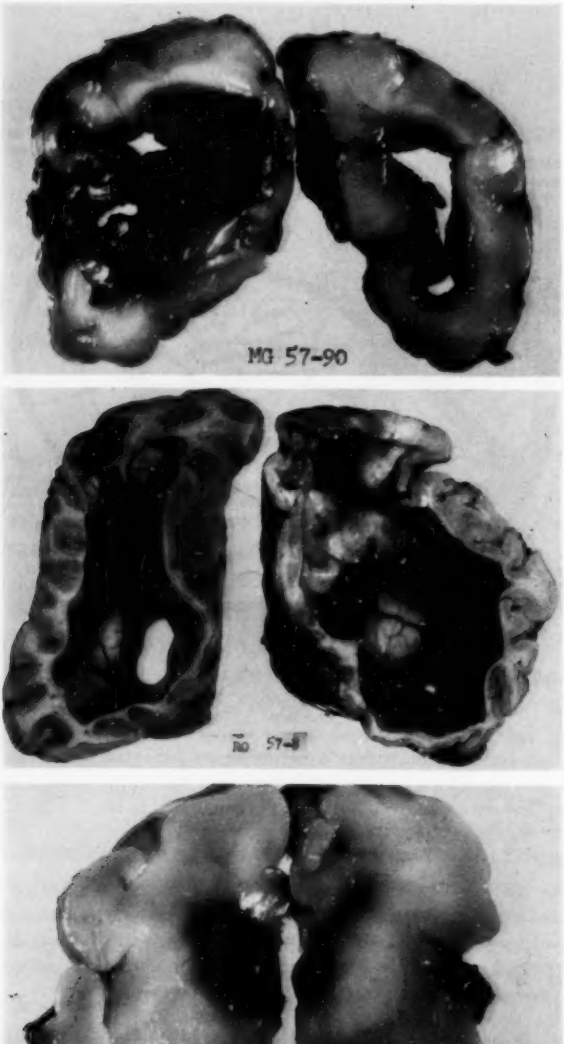

1
2
Rupture sup. cerebr. vv. with subdural hematoma of convexity or generalized subdural hemorrhage
3
Rupture gr. cerebr. v. (Galen) with subtentorial hemorrhage at base of brain
Hemorrhage from terminal v. or choroid plexus with extension into ventricles and subarachnoid space
5
Rupture at junction of falx and tentorium
Isolated hemorrhages in pia mater
Sup. sagittal sinus
Sup. choroid v.
Inf. cerebr.
IIIrd vent.
Subependymal hemorrhage with subsequent rupture into lateral ventricles
Mid. cerebr. v.
4
Post. cerebell. vv.
Tentorial lacerations medial into straight sinus or lateral into transverse sinus — subtentorial or generalized hemorrhage
Traumatic
Anoxic

FIG. 2. (Haller, Nesbitt and Anderson). Diagram of the intracranial dural and venous relationships showing frequent sites of injury and common sources of hemorrhage. Anoxia-induced lesions are indicated by numbers encircled in black and traumatic lesions are depicted by numbers encircled in white.

Case 2. Baby girl R. (M.G. 57-90) was premature, weight 3 lb. 1 oz. Delivery was spontaneous. Infant seemed normal at birth. Five hours later cyanosis developed and breathing ceased for several minutes. During the remainder of the first day of life there were frequent periods of cyanosis. On the second day there were convulsions with rolling up of the eyes, and opisthotonos. Death occurred on the third day.

Comment Case 2 Figure 3. The large hematoma in the left temporoparietal region has destroyed almost all the white matter including the left optic radiation. The hematoma was accompanied by hemorrhage into the leaflets of the tentorium bilaterally indicating that a major deformity of the head had occurred. The 5-hour period of seeming normalcy suggests that the hemorrhage enlarged during this time, then exerted pressure on the midbrain and brain stem with resultant respiratory distress


MG 57-90
WH 59-42

and cyanosis and later convulsions. The rolling up of the eyes and the opisthotonos suggested further pressure on the midbrain region.

Case 3. A.B. (313-57), a 37-year-old man was blind from birth or a very early age. Six weeks after birth he suffered from severe convulsions. The lowers limbs, at first spastic, became atrophic. The head was slightly enlarged. The eyes were said to have been normal as regards external appearances and optic fundi.

Comment Case 3 Figure 4. It seems probable that in this individual bilateral intracerebral hemorrhages occurred during the first weeks of life and these were responsible for the cysts seen on the outer aspect of each lateral ventricle. The ventricular dilatation probably resulted from obstruction of the aqueduct. Certainly both optic radiations were destroyed. There is similarity to Case 2 Figure 3.

Case 4. Baby D. (59-42) was premature infant, 30 weeks gestation. Labor was normal. The amniotic fluid was clear. Fetal heart rate was 90 per minute. Partial breech extraction. Apnea was present for 7 to 8 minutes. Oxygen was administered for 15 minutes. Ecchymoses were present on right thigh and leg. There was generalized edema. Respirations were at first abdominal, then of the Cheyne-Stokes variety. Death occurred 60 hours after birth.

Comment Case 4 Figure 5. In this case Figure 2 is of interest. Anastomotic veins along the lateral ventricles are frequently the site of hemorrhage which may extend into the brain tissue and may rupture into the ventricles. Also it is of particular interest that the left parietal bone was depressed at the time of autopsy and the tentorium showed laceration. Such a lesion as this does not directly involve the visual pathways but pressure and edema produced by the hemorrhage within the tissues may secondarily involve the visual pathways as for example the lateral geniculate body (see Fig. 6B).

Case 5. A.W. (338-54). This boy died at 15 years of age. The history was incomplete. Birth was said to have been normal and the pregnancy was uncomplicated. However he

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Fig. 3. Baby girl R. (MG 57-90). A large hematoma is seen in the left temporoparietal region. The ventricle wall is destroyed and there is loss of white matter. There is similar but less severe damage in the right hemisphere.

Fig. 4. A.B. (313-57; 57–8). Coronal section through the parietal regions shows pronounced dilatation of the lateral ventricles. Both posterior ventricular horns are separated from the ventricle wall by a cyst. The cysts outside the ventricles probably originated from intracerebral hematomas. Bleeding into the ventricles produced granulation of the ependyma in the aqueduct followed by hydrocephalus. Both optic radiations are destroyed.

Fig. 5. Baby D. (59–42). Hemorrhage into left lateral ventricle and caudate nucleus. There was laceration of the tentorium and the left parietal bone was depressed.

was in an incubator for two weeks after birth. He had convulsive seizures at 2 years. There was generally defective physical development and mental deficiency. Information as regards the eyes and visual acuity was not available. The diagonsis was birth injury. A pneumoencephalogram showed evidence of cerebral atrophy, more pronounced on the right.

Comment Case 5 Figure 6. The cystic lesion in the white matter of the right hemisphere resulted from traumatic hemorrhage. The atrophy of the right thalamus, of the cornu ammonis, and right lateral geniculate body in which only a few cells survived, probably originated in vascular compression when the hematoma was fresh and space-consuming. Interruption of thalamocortical fibers also contributed to depletion of nerve cells in the thalamus. Sclerosis of the lateral geniculate body, the cornu ammonis and adjacent thalamus occurs relatively frequently when there is pressure on the brain at the edge of the tentorium. We have evidence that it occurs at birth and later in infancy as will be shown subsequently.

Case 6 Baby D. (55-65), a full-term male infant was delivered by caesarian section after an effort to deliver with forceps had failed. Infant cried 10 minutes after delivery. Respirations became irregular and shallow and the infant became pale. Death occurred 30 hours after birth.

Comment Case 6 Figure 7. It has been remarked earlier that birth injury may result from compression of the dural sinuses or cerebral vessels, principally veins. In the patient described above the parietal bones had overlapped. This compressed the longitudinal sinus in the centroparietal region obstructing the outflow of the dorsal veins. This in turn produced stagnation of blood circulation in the cortex at the convexity of both cerebral hemispheres as well as their medial aspects, with resultant softenings involving the dorsal portions of both cerebral hemispheres and of the cortex facing the falx. The ventral portions of the gyrus cinguli have remained intact. The lesions seen in this case do not directly involve any portion of the primary visual pathways. However, as is shown in the next case (Case 7) the same mechanism may produce lesions which involve the calcarine areas.

Case 7. Baby G. (55-12). This baby died at 3 years of age. The child exhibited quadriplegia which was attributed to birth injury.

Comment Case 7 Figure 8. In the section showing the brain from above, it is seen that there is atrophy of the anterior and posterior central convolutions as well as the superior portions of the parietal lobes. This resulted from an obstruction of the venous drainage through the middle third of the superior longitudinal sinus with consequent hypoxia (Fig. 8A). How-

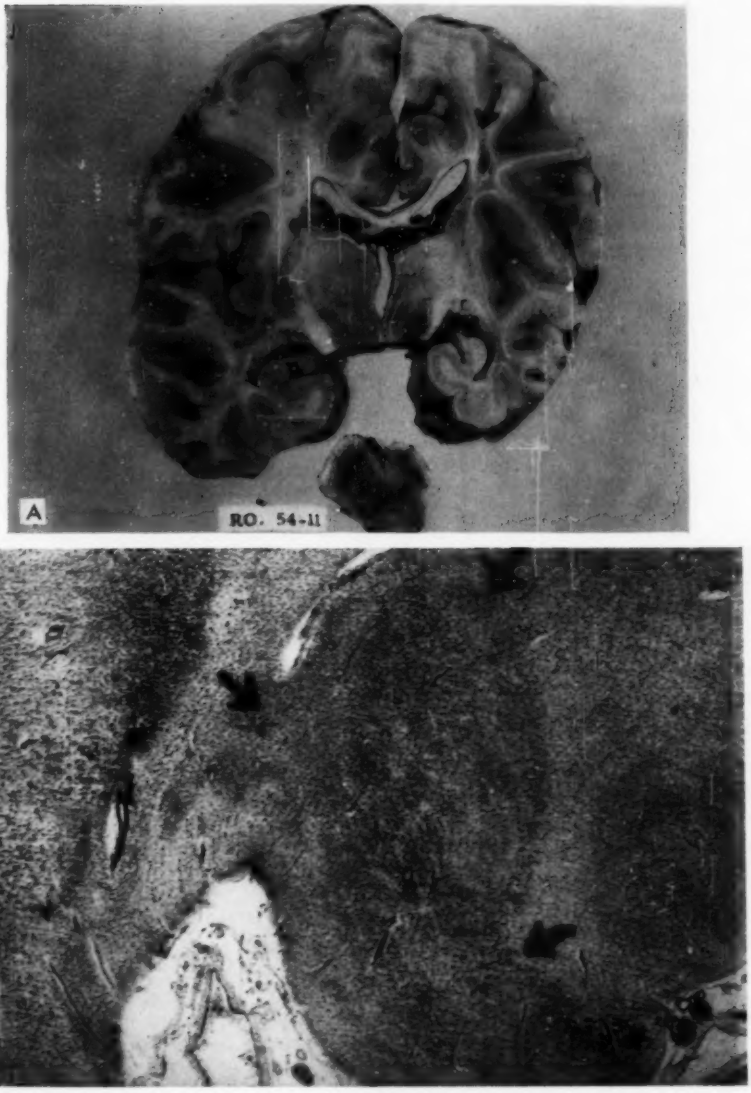


FIG. 6. A.W. (338-54). A (*top*). The right hemisphere is small. The thalamus and cornu ammonis are small; the thalamus gray. A cyst in the right hemisphere is indicated by arrow. B (*bottom*). Loss of nerve cells in the right lateral geniculate body. Ammon's horn not shown. Loss of cells is assumed to be due to pressure rather than to nerve or tract lesion because of absence of lamination in lateral geniculate body.

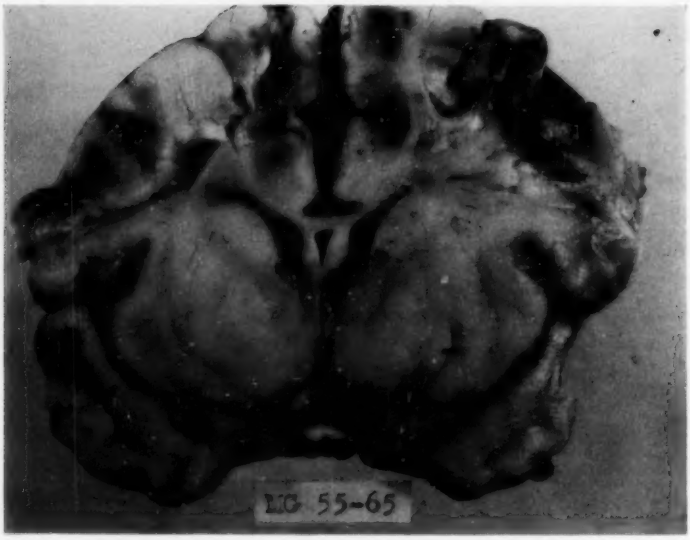


FIG. 7. Baby D. (55-65). There is pronounced destruction of the dorsal portion of both cerebral hemispheres. Both contain hemorrhages. Overlapping of the parietal bones and obstruction of the dorsal veins draining into the longitudinal sinus has been responsible.

ever, there is extensive damage posteriorly. Probably the situation as seen in Figure 8B resulted from compression of the superior longitudinal sinus in its posterior third interfering with drainage to dorsal veins in that area. The atrophy or sclerosis involving the calcarine areas and the adjacent cortex around the depths of the convolutions, is due in part to swelling and edema which occurred when the principal lesion in the central cortex was acute.

Obstruction to veins and venous sinuses accounts for brain damage more often than obstruction to arteries as part of cerebral edema and swelling. It seems probable that the following case report exemplifies arterial involvement.

Case 8. D.M. (59-37), a 25-year-old female was born as a breech presentation. Birth was difficult. For 3 months she appeared lifeless and was unable to move her limbs. Later there was spastic quadriplegia and inability to move voluntarily. She never learned to talk. She suffered epileptiform seizures and was severely mentally retarded.

Comment Case 8 Figure 9. The loss of tissue in the left parieto-occipital region may have resulted from softening in the supply area of the middle cerebral artery, with involvement of the parietal, posterior temporal and angular branches and sparing of the orbital and frontal branches. Pos-

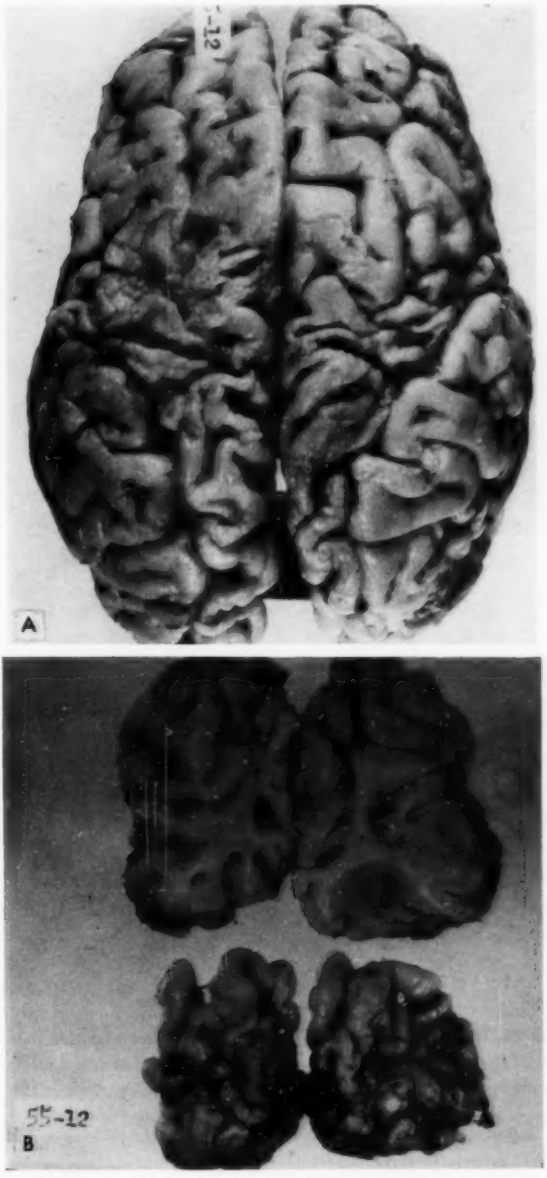


FIG. 8. Baby girl G. (55-12). A. (*top*). Bilateral sclerosis central convolutions and upper parietal lobes probably due to obstruction venous drainage as a result of thrombosis or compression of superior longitudinal sinus. B (*bottom*). Both calcarine areas destroyed. Severe atrophy probably resulted from pressure.

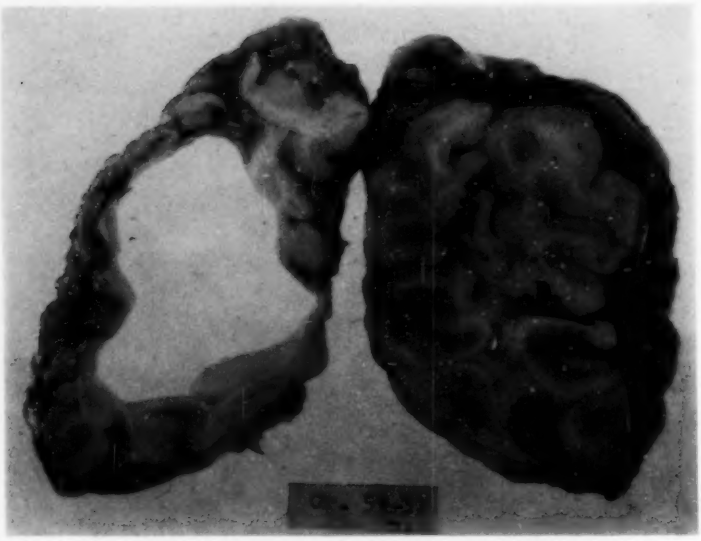


FIG. 9. D.H. (59–37). Section through the parietal lobes. There is pronounced loss of tissue left parieto-occipital region and minimal loss of cortex in the deep right parieto-occipital lobe.

sibly overlapping of the bones in the area of the temporal squama was responsible for pressure on the branches of the middle cerebral artery, and it is likely there was venous compression as well. The optic radiation on the left is completely destroyed and thus hemianopsia was present on the right. The damage to the left hemisphere in this case is similarly located to the fresh traumatic hemorrhage shown in Figure 3. The damage to the right parieto-occipital lobe probably resulted from pressure.

The case report which follows has seemed to us as particularly valuable as regards factors which may influence the occurrence of cerebral birth trauma. Here the sequence of events as outlined seems reasonable.

Case 9. G.T. (58-20) a boy, died at 10 years of age. Birth was at term, weight 8 lb. 6 oz. Birth lasted 56 hours and was difficult. The child was an idiot, could not walk, talk, feed or dress himself. He was incontinent. There was generalized spasticity most pronounced in the upper right extremity. The pupils were eccentric but reacted to light. There were searching movements of the eyes. The optic discs were chalky white.

Comment Case 9 Figure 10. The large smooth walled cyst in the left upper parietal region Figure A, probably originated in intracerebral hematoma at the time of birth. (see Figs. 4 and 6). As a result of the hemorrhage, swelling and softening of the tissues in this region accounted for the ex-

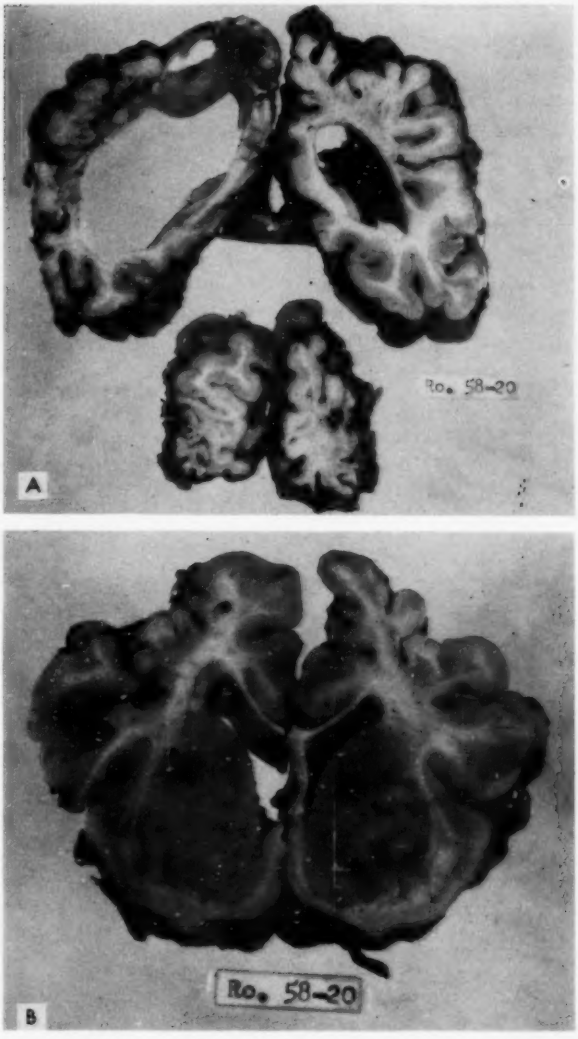


FIG. 10. G.T. (58-20). A (*top*). Section through parietal and occipital lobes. Pronounced loss white matter. Cyst upper left parietal lobe probably originated in hematoma at birth. Dilated left posterior ventricular horn probably due to shrinkage of tissue in temporo-parietal region. Both calcarine areas destroyed as a result of pressure and tissue necrosis. B (*bottom*). Section at level head of caudate nucleus. Bilateral scarring second frontal convolution. Extensive destruction white matter.

tensive loss of tissue and the large left posterior ventricular horn. Also in Figure 10A the sclerosis of the upper right parietal lobe may have partially originated in venous compression.

Particularly in Figure 10B there is evidence of damage to the border zone area bilaterally between the anterior and middle cerebral arteries. Such border zone distribution is also present in the border area between the anterior, posterior and middle cerebral circulations posteriorly (Fig. 10A). This type of involvement is observed in the hypoxia associated with temporary heart failure. In this case it seems reasonable to suggest that after intracerebral hemorrhage developed there was a drop in blood pressure amounting to temporary heart failure. Such a factor must be important in determining the cerebral effects resulting from birth injury.

SUMMARY REGARDING LESIONS DUE TO DEFORMATION OF THE HEAD DURING BIRTH

Lesions which directly injure the brain due to deformation of the head usually are unilateral. They may primarily involve the optic pathways or may damage them secondarily through vascular compressions during the early phases of the traumatic involvement. Indirect birth injuries are bilateral frequently, and predominantly involve the convexity of the cerebral hemispheres. Swelling of the brain may produce involvements of the primary visual pathways including the calcarine cortices as a complication of temporary heart failure with consequent hypoxia.

PROLAPSE OF THE UMBILICAL CORD

Dr. Irvin Cushner (13) of the Department of Obstetrics, Johns Hopkins Hospital, examined all case records of prolapse of the umbilical cord from 1896 to 1956. During the 60-year-period, 87,963 women were delivered, and prolapse of the cord occurred in 424 instances. Information was obtained on 66 of 152 fetal survivors. Two individuals were dead; 1 had suffered from sickle-cell disease, and the cause of death was not known in the other. One individual suffered from diabetes. Three children who were located were not normal. One, a Negro male 11 years old, was delivered by version and extraction less than 30 minutes after prolapse of the cord was recognized; birth weight was normal. School performance was at a 5th grade level instead of 9th. Another, a Negro child 8 years of age, was delivered by breech extraction less than 30 minutes after prolapse was noted; weight was less than 1,000 grams. There was mental and physical retardation and retrolental fibroplasia when examintaions were made. A 3-year-old child delivered 30 minutes after the prolapse was recognized had a birth weight of 1,000 to 1,500 grams. The child was mentally and physically retarded and exhibited spasticity.

Cushner's conclusions seem reasonable. (1) Permanent cerebral damage is a less common sequel in fetal survivors of prolapsed umbilical cord than was thought. (2) Prematurity well may be a more valid explanation for residual damage than cord prolapse. However, from the figures given it is apparent that prolapse of the cord often accounts for death. Particularly in premature infants accurate assessment of the influence of a prolapsed cord is extremely difficult. There is not any cerebral finding which is characteristic of compression of the cord. Probably compression of the cord results in an extra burden on the fetal heart and cardiac inadequacy produces cerebral changes and compression of vessels with subsequent necrosis of cerebral tissues if death does not occur. This leads to consideration of temporary heart failure in newborn infants and young children.

TEMPORARY HEART FAILURE

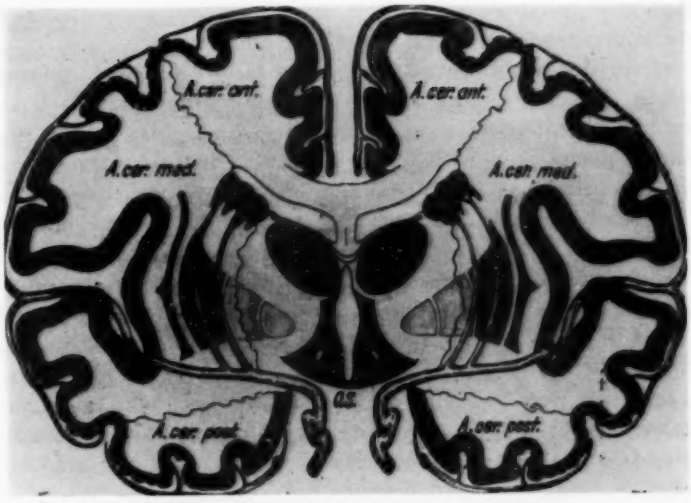
A decrease in the pulse rate of the fetus is not unusual during the birth process. However, little is known concerning temporary heart failure *in utero*. Cooke (14) in his studies on sheep fetuses, found that with induced anoxia there was at first heart block which later disappeared. Windle in his anoxia experiments on monkey fetuses observed at first an increase in the number of heart beats followed by an appreciable decrease (personal communication). Temporary heart failure in the fetus may occur without obvious reason as has been observed by Dr. David Clark (15). The recently introduced technique of recording EKG's on the fetus before and during birth should provide more information regarding transient heart failure in this period.

Temporary heart failure leads to stagnation of circulation. The defective circulation first involves the peripheral portions of the arterial supply, and therefore the cerebral lesions which develop predominantly are situated in the areas of border zone circulation from the large cerebral arteries (Fig. 11).

In cases of temporary cardiac arrest the principal cerebral damage is in the gray matter, particularly the cerebral cortex. The distribution of the cerebral lesions is characteristic in that they are most severe in the terminal supply areas of the major brain arteries, and gradually decrease toward the larger branches and stems of these arteries. The border zones between the parietal branches of the anterior and middle cerebral arteries bilaterally usually are involved first. In more prolonged cardiac arrest, the lesions extend over the upper central region into the frontal lobes, and then also involve the border zones between the posterior and anterior and middle cerebrals and thus the occipital lobes involving the calcarine areas are involved. In such severe cases the caudate nuclei, putamina and usu-

ally the thalami are affected. The cortical distribution pattern of the lesions suggests that during the fall in arterial blood pressure the most distal branches of the largest and longest cerebral arteries are the first to develop capillary stagnation. When heart action returns the blood pressure builds up gradually to normal levels, consequently stagnation in the more distal arterial border zones will persist longer than proximally. The alterations in the second frontal convolutions of both hemispheres (Fig. 10B) are characteristic residuals of temporary capillary stasis due to severe fall in blood pressure or temporary heart failure. This is in agreement with Meyer (16), also Norman *et al.* (17).

Personal observations (5) on a large number of cases of temporary cardiac arrest suggest that the cerebral damage is limited to the gray matter if the left heart fails whether or not there is coincident failure of the right heart. Major involvement of the left heart occurs in hypoxemias of various types, excepting those caused by obstruction of the pulmonary circulation. If the hypoxia is caused by impairment of the pulmonary circulation as in incomplete expansion of the lungs, acute lung edema, interstitial pneumonia etc., the right ventricle tends to fail before the left ventricle. Then, it seems reasonable that with the return of blood to the right heart severely retarded, the venous blood pressure is increased producing a congestion of veins. Such congestion will be present in the white matter of the cerebral hemispheres where long veins extend from the cortex to the lateral angle of the lateral ventricles from whence the blood is drained by a horizontal longitudinal anastomosis and terminal veins into the internal cerebral vein, then into the great vein of Galen, and into the straight sinus. Since with right heart failure some degree of failure of the left heart is associated it would be anticipated that the venous congestion within the white matter would be more pronounced in the parieto-occipital region of the hemispheres than frontally for several reasons now stated. As the force of the left heart becomes weaker the pressure in the peripheral arterial branches of the longest arteries (occipital and parietal) decrease more than in the peripheral branches of the shorter arteries (frontal). Therefore, in the anterior half of the brain the blood still will be pushed through the capillary network and thus afford some venous drainage. In the parieto-central region the tendency to venous stagnation will be greater than anteriorly, and in the parieto-occipital region it will be greatest. If venous stagnation develops rapidly, there is development of tissue necrosis. In contrast with necrosis of the gray matter, necrosis in the white matter tends to be deep. The white matter of the parietal lobes belonging to the arterial border zones is likely to be involved relatively early and as the circulatory deficiency advances there will be development of necrosis in the white matter of the central and frontal regions. Admittedly this concept must be considered as not estab-



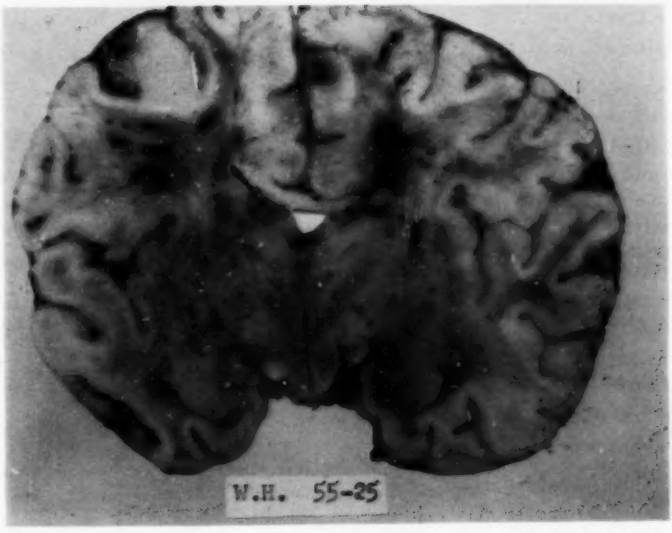


FIG. 11. (Lindenberg). Diagram illustrating supply areas of the three major brain arteries. The terminal portions of these vessels are shown in solid black. Adjacent to the solid black areas of arteries there are situated the areas of most pronounced stasis in temporary heart failure. Irregular contours of the adjacent gray matter indicate the most severe tissue damage in the cortex, also in the basal ganglia. Z. N. 1939.

FIG. 12. Baby girl W. (55-25). Section at level of tuber cinereum, posterior clinoid processes. Note large cavum pellucidum. Severe swelling of the brain with edema and small hemorrhages around veins in the white matter. Compression of ventricles and sulci. Also there was shown histologically widespread necrosis in the occipital lobes and in the deeper portions of the white matter particularly in the centrum ovale region.

lished beyond question but what is seen at autopsy suggests that the sequence of events as outlined is reasonable and logical.

In considering the influence of cardiac failure on the cerebrum it is interesting to know that the effects of hypoxia on the heart have been studied. In systemic hypoxias, regardless of their origin, one exposure may lead to necrosis of muscle fibers of the left heart; these areas of necrosis are characteristically located close to the endocardium and within the papillary muscles. However, it has been shown that in some instances the right heart takes the brunt of damage. This occurs if the pulmonary circulation is impeded by improper inflation of the fetal lungs, by pulmonary embolism or by interstitial pneumonia. In such cases the necrosis of muscle fibers is most pronounced near the endocardium of the right ventricle (see Büchner *et al.* (18)). Considering the nature of the cardiac involvements resulting from hypoxia, it is suggested that lesions in the arterial border zones predominantly involve the gray matter of the cortex and the striate bodies and are caused principally by failure of the left heart (see Fig. 11).

The severe swelling of the brain especially of the white matter seen in Figure 12, probably resulted from heart failure predominantly right-sided.

Case 10. Baby girl W. (55-25) because of placenta previa was delivered by caesarian section. Birth weight was 8 lb. 12 oz. Appearance at birth was normal but there was no spontaneous breathing. Oxygen and positive pressure administered, and respirations were established in 5 minutes. The infant was placed in Bloxom apparatus and 3 hours later breathing was normal. The infant was flaccid. Four days after birth infant died from pneumonia.

Comment Case 10 Figure 12. Severe swelling of the brain with edema and small hemorrhages about the veins is seen in the centrum semiovale regions. Histological studies showed widespread necrosis in the deeper portions of the white matter, particularly in the region where hemorrhages were pronounced, and also widespread necrosis of the occipital cortex.

Case 11. P.S. (59-40) died at 6 years of age. The child was born at term, weight 5 lb. 4 oz. At 8 months had convulsions. He was mentally retarded. It was impossible to obtain further information other than that he was quadriplegic.

Comment Case 11 Figure 13. These figures show what might be considered as an end result to what is shown in Figure 12. The loss of white matter in both cerebral hemispheres originated from tissue necrosis and hemorrhages which had occurred in the area centrum semiovale. The loss of cortex around the depths of the sulci probably resulted from vascular compression within the sulci during the period of severe brain edema. Further posteriorly the white matter damage, also the cortical

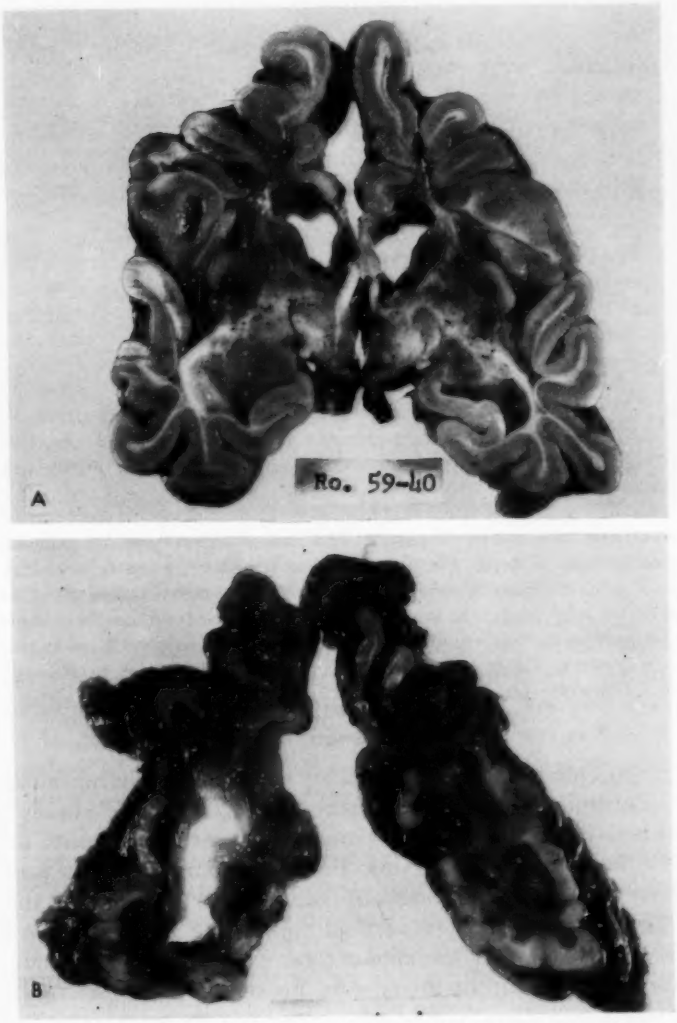


FIG. 13. R.S. (59-40; 1160). A (*top*). Section at level tuber cinereum. Cortex around sulci missing. Extensive destruction white matter. (Probably represents right heart failure). B (*bottom*). Occipital lobes show complete loss of white matter and both calcarine areas destroyed, probably by pressure as well as necrosis.

damage, is most pronounced in the occipital regions and undoubtedly was responsible for blindness in this child.

Cases 9, 10 and 11 exemplify severe and extensive cerebral damage resulting from temporary failure of the heart. In survivors with less severe

damage, cerebral involvement limited to the parieto-occipital region does not of necessity involve the primary visual pathways, but may do so as a result of vascular compression.

A case is now briefly described because of its intrinsic interest; in it involvement of the higher visual pathways is important, but this type of involvement is not under consideration here. The case is included because it well may have resulted from temporary heart failure.

Case 12. M.N. (875098), a white woman 21 years of age had convulsions at birth. She seemingly developed normally. Her eyes had been examined on several occasions and were thought to be normal until she was seen by Dr. Richard Hoover who kindly allowed us to see her. Visual acuity was 20/200 and 20/20 for the right and left eyes respectively. The optic fundi were normal. There was a low-grade alternating internal strabismus and it was thought that the low vision in the right eye was due to amblyopia ex anopsia. Both lower visual fields were lost. The patient was intelligent with a full scale I.Q. of 109 and verbal I.Q. 135; nonverbal I.Q. 74. She had a disability for space and form perception and loss of visuo-motor skills so that her handwriting was uncontrolled and she was unable to draw or do any type of needle work. She had been utterly unable to learn to type.

The disability as regards space and form perception and optico-motor coordination indicated bilateral damage to the parietal and parieto-occipital regions probably occurring about the time of birth. Possibly there was temporary heart failure. Loss of the lower visual fields indicates bilateral lesions involving the dorsal radiations or the dorsal calcarine cortex areas. Such field loss well may have resulted from compression of arterial branches supplying the affected areas during the phase of brain swelling. In this regard attention is drawn to Figure 14 where trauma provided compression necrosis of the calcarine areas bilaterally but spared the upper lip of the calcarine on the right.

HYDRANENCEPHALY

Hydranencephaly is a rare abnormality in which the external appearance of the head and eyes does not betray the severe loss of tissues in the cerebral hemispheres. The loss of tissue is the result of extensive necrosis. Spatz (19) has shown that necrotic tissues are removed faster and more completely in the immature nervous system of the fetus than in the adult brain. The autopsy may show such an appearance as that seen in Figure 15. It is remarkable that the almost total damage to the cerebral hemisphere is in sharp contrast to the contents of the posterior cranial fossa where only a secondary hypoplasia is observed. In such cases the head may appear quite normal and the early activities of the infant such as crying, sucking and sleeping may be normal. Early evidence of the affection may be failure of optical responses to stimuli so that observant parents are worried as to the possibility of blindness. Poikilothermia may be the reason for seeking advice. In such a case a flashlight held against one temple shines through to the opposite side since the hemispheres are replaced by fluid. Pupillary responses may be present. Some portions of the striate

FIG. 14. (312-52; 52-970). Adult brain. Compression necrosis calcarine branches of posterior cerebral artery on both sides. On the right the upper lip of the calcarine remains relatively normal. A trauma case with subdural hematoma.

bodies, the pallida, and particularly the thalami and cortex of the lower temporal lobes and occipital poles tend to be preserved because these areas are supplied by the posterior cerebral arteries. However, these areas also may be destroyed when the posterior cerebral arteries have received their supply of blood via the carotid arteries, or when the posterior cerebral arteries have been compressed at the tentorial edge because of cerebral swelling.

The preservation of the contents of the posterior fossa, supplied by the vertebral and basilar arteries, suggests that the cerebral damage is produced by temporary circulatory deficiency in both carotid arteries. This might occur when the umbilical cord is wrapped around the neck during intrauterine life. In such an instance the jugular veins are compressed before the carotid circulation is involved, but venous compression in itself would not explain the tissue loss. It has been stated by one of us (Lindenberg) that when there is severe swelling of the fetal or infant brain the carotid arteries may be pressed against the sphenoid bones if the blood pressure is lowered. Also when necrosis develops within the hemispheres the pressure increases and this leads to compression of the carotid arteries. Manual strangulation of the newborn may produce essentially what has been described.

Case 13. Baby girl H. (57-281) was a first child, birth weight 6 lb. 7 oz. Birth was uneventful. Respirations were spontaneous. The head was small. The child was pale and gray. There was absence of cranial sutures except for rudiments of them in the occipital region. There was progressive loss of weight. Death occurred two weeks after birth. The eyelids and eyes were said to have had a normal appearance but no mention was made of pupillary responses. Sections showed pronounced atrophy of the ganglion cells. The other retinal layers seemed remarkably well developed. The optic nerves showed extensive gliosis and absence of myelination.

II. INVOLVEMENTS OF THE PRIMARY CEREBRAL VISUAL PATHWAYS IN INFANCY AND CHILDHOOD

The cerebral lesions observed in infants and children who were normal at birth and enjoyed a period of normal development are similar to those observed in newborns excepting that birth injuries are substituted by mechanical injuries. However, there are important differences. In the immature brain necrotic tissue or blood clot is removed rapidly and completely. The resultant cyst formation usually is smooth-walled and it may be impossible to decide if it resulted from necrosis or hemorrhage. In the more mature brain the removal of necrotic tissue or blood clot is accomplished more slowly. In an area of softening, vascular connective tissue often remains filling the resultant cyst with a spider-web network of vessels. During resorption of a hematoma the connective tissue may produce firm scars.

It is generallly well known that the brains of infants and children are particularly liable to severe swelling. As a result lesions originate in vascular compression. Swelling of the brain results from shock accompanying trauma, from infectious diseases or from chemical or thermal injuries. What seems an inconsequential blow to the head may shortly afterwards result in shock and during the period of shock the brain may swell severely. As an example we may cite the case of a young child who died 30 minutes after a relatively mild blow to the head; respiratory failure was produced by severe pressure on the hypothalamus and midbrain regions. If the shock is survived, cerebral lesions which may have developed are produced by one of two mechanisms. (1) Stasis in the periphery of the arterial end-zones as already described produces symmetrical lesions involving the border zones of the larger brain arteries. Consequently the occipital lobes are the site of most severe damage (see Case 14 Figure 16). (2) Vascular compression accounts for lesions in areas of predilection (5C). Such lesions resulting from vascular compression occur more frequently in infants and children than alterations in the arterial border zones but occasionally they may be associated. Vascular compression lesions involve the primary visual pathways as is illustrated by Case 5, Figure 6B, is also examplified by Case 15 Figure 17. Combined involvements of the cornu

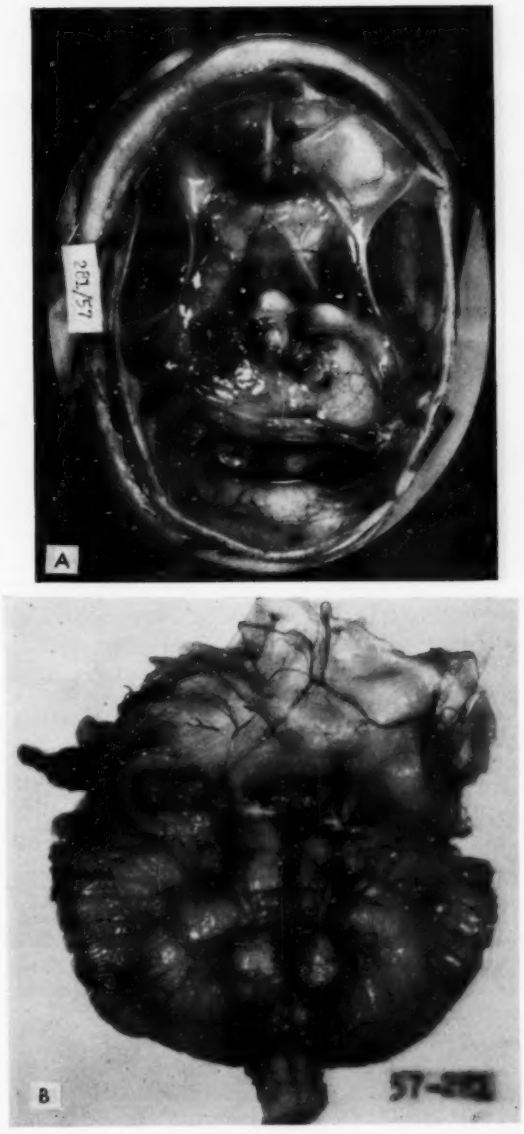


FIG. 15. Baby girl H. (281-57). Hydranencephaly. A (*top*). Looking down on the fully formed cerebellum and deformed midbrain. Some remains of hippocampus bilaterally. Cerebral hemispheres absent. Glial membrane at base represents the former superficial layer of cortex still adherent to the arachnoid. Note the cysts behind the cerebellum. B (*bottom*). Glial tissue membrane in the region of the basal cistern and vicinity. The anterior portions of the circle of Willis are seen readily (carotids with branching into the anterior and middle cerebrals and the anterior communicating artery). The cerebellum and brain stem showed only a secondary hypoplasia. Absence of hemispheres accounts for absence of pyramids.

Necrosis of the cerebral hemispheres occurred during fetal life because the stump of midbrain, A, shows malformation resulting from immature nerve and glial cells growing over the margin of the defect.

FIG. 16. Baby J.H. (343-54; 54-641). The occipital lobes showed extensive hemorrhagic necrosis of cortex. Note thin subarachnoid hematoma. There was absence of traumatic brain lesions.

ammonis, lateral geniculate body and thalamus doubtless originates in compression of vessels in the lateral portion of the cisterna ambiens. We have observed rather often such a triad of lesions as resulting from trauma during or after birth, also when there has been no history of trauma (Case 15, Fig. 17).

Case 14. Baby J.H. (343-54) was a 2-year-old boy who was beaten by his grandmother, became unconscious and survived for 2 days. The autopsy findings suggested that there was profound shock and that the brain lesions resulted from border zone involvement.

Case 15. P.M. (53-2), a 4-year-old child was struck by an automobile. A small subdural hematoma was found over each hemisphere, and the brain was "tight". Death occurred 2 days after the injury.

Case 16. J.G. (13784.A27849), a boy, died at 4 years. There was nothing remarkable about the birth and development was normal until the child was 4 months old. Then he developed diarrhea and was severely dehydrated. Thereafter development was defective and from 15 months to the time of death there were convulsive episodes and the child was quite blind. There was optic atrophy bilateral. Terminal pneumonia accounted for death.

Comment Case 16 Figure 18. At the autopsy it was observed that there was atrophy of the cerebral hemispheres most severe over the frontal and parietal lobes with relative sparing of the temporal lobes and sparing of the posterior occipital lobes. The cerebellum brain stem and cord seemed normal externally in contrast to the shrinking of the hemispheres. Mi-

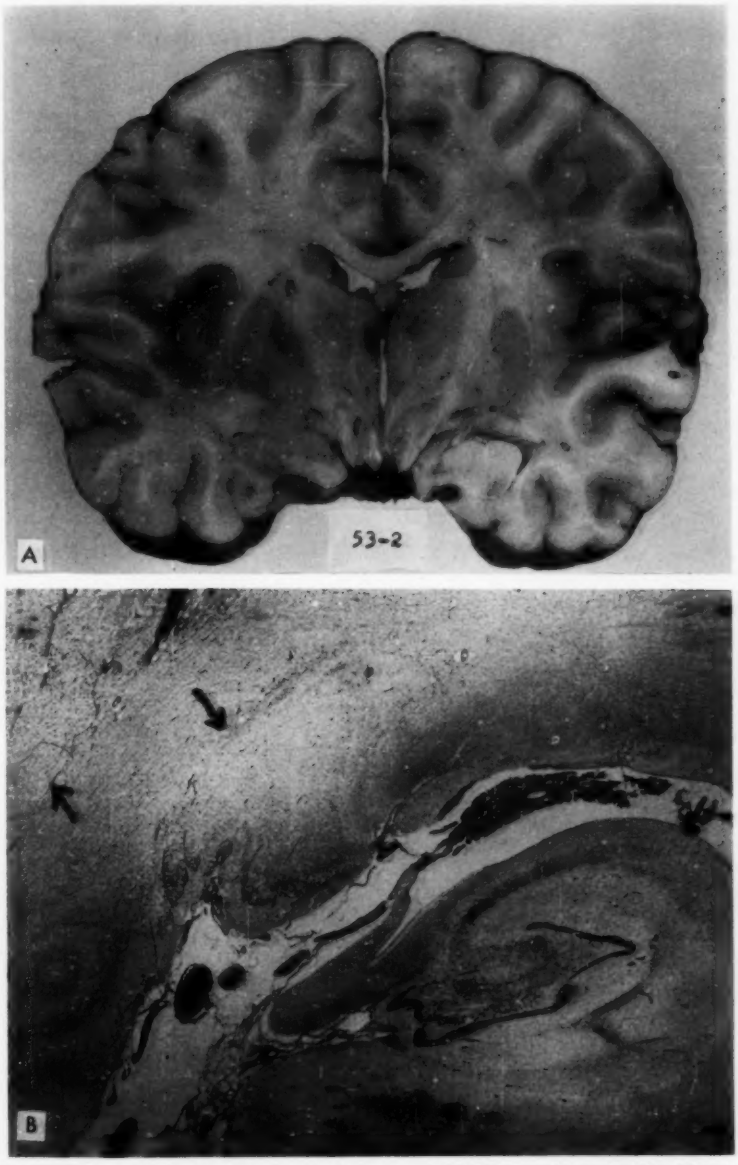


FIG. 17. P.M. (1-53; 53-2). A (*top*). Section at level of the mamillary bodies. The brain is severely swollen. Contusion hemorrhages are visible in both unci. Hemorrhage in the right hemisphere is due to a neurosurgical procedure. B (*bottom*). Early necrosis of the lateral geniculate body and adjacent Ammon's horn result of compression of veins and arteries and swelling in this region.

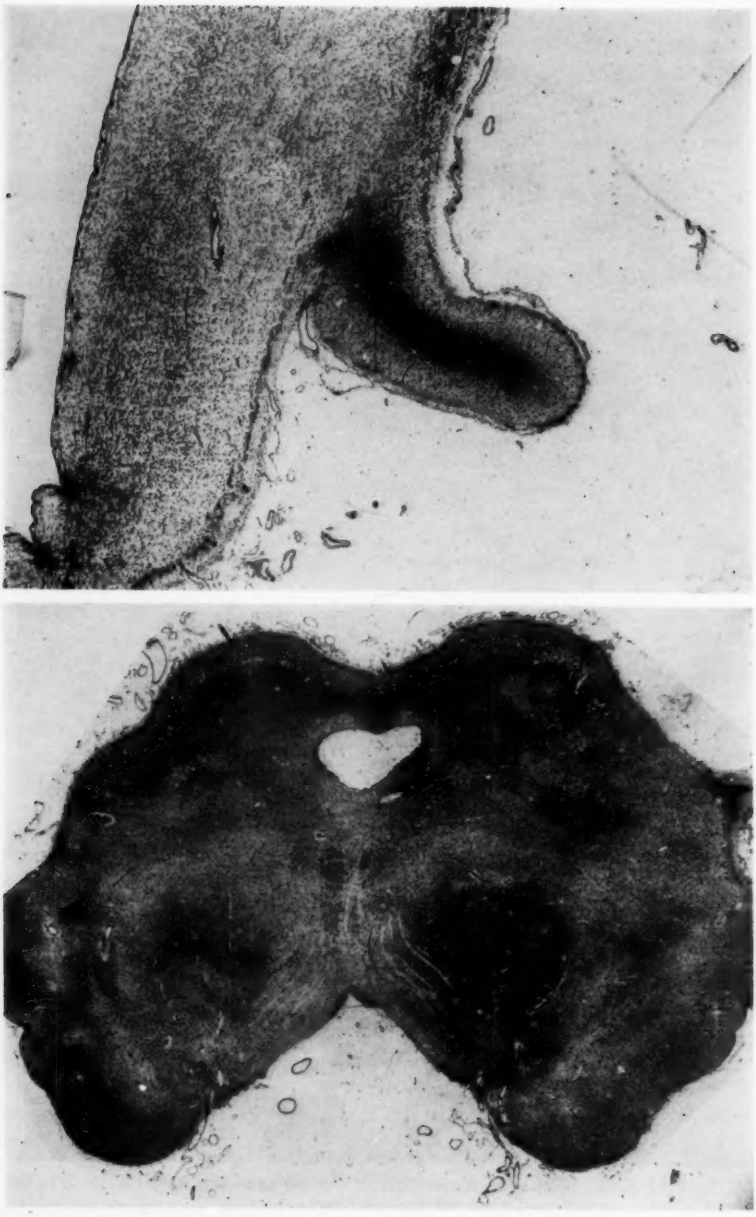


FIG. 18. J.G. (H.L.H. B13784). A (*top*). Astrocytic scarring central optic tract associated with loss of myelin sheaths. Level tuber cinereum. B (*bottom*). Lesions in mesencephalon with pronounced involvement superior colliculi and one red nucleus.

croscopic studies showed extensive loss of cortical neurones excepting in the posterior occipital region.

The sections selected for presentation here illustrate damage which would explain ocular symptomatology.

Dr. David Clark who studied this case and made it available to us explains the cerebral changes on the basis of brain swelling due to vascular insufficiency associated with hypoxia resulting in pressure. With Dr. Clark we have studied another case (H.L.H. B 59302. aut. 28876) with almost identical findings to those recorded here as Case 16 Figure 18; figures from autopsy 28876 are not reproduced here but they also duplicate what is seen in Case 15 Figure 17.

Brief recapitulation at this point serves to emphasize the importance of pressure changes which are associated with swelling and produce involvements such as described in Case 15 Figure 17 and Case 16 Figure 18. We have observed now in many instances coincident involvement of the cornu ammonis, lateral geniculate body and thalamus on such a basis. It might be anticipated that pressure in this general area might produce variable effects. Such variability is shown in Figure 19. In it the lateral geniculate body is preserved and the medial geniculate body is severely damaged.

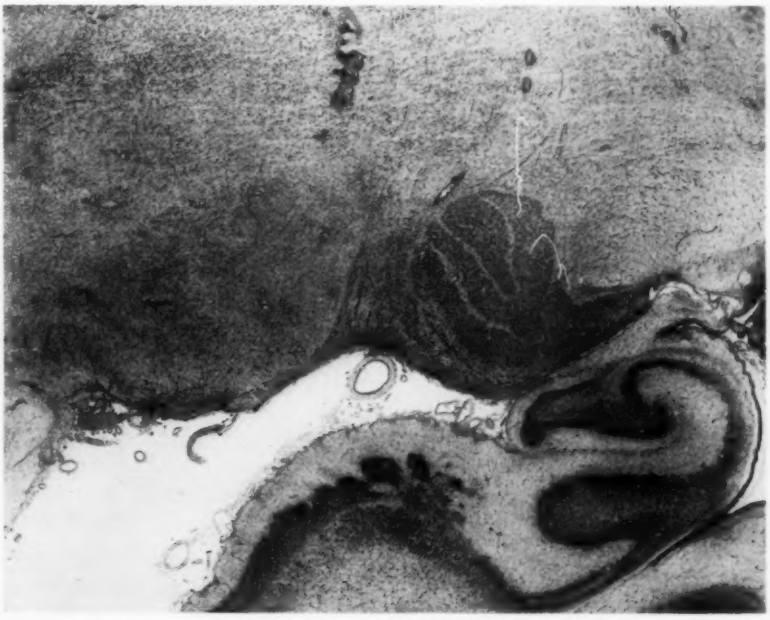


FIG. 19. Prolapse of cord in newborn infant. The lateral geniculate body is preserved. The medial geniculate body is damaged. (Courtesy Dr. D. B. Clark.)

Vascular insufficiency associated with hypoxia and swelling resulting in pressure accounts for involvements of the primary visual pathways throughout. Particularly with trauma swelling of the optic nerves and chiasm is observed frequently. Similarly swelling and traction on the tract (Fig. 17A) is seen not infrequently. Pressure changes in the lateral geniculate body are shown (Figs. 17 and 18). The calcarine cortex is damaged by pressure on the branches of the posterior cerebral arteries along the edge of the tentorium. All such involvements may result in atrophy and sclerosis.

In some instances only the calcarine cortex, unilaterally or bilaterally, is the site of cellular necrosis and subsequent sclerosis. Figure 20 is of interest in this regard. It exemplifies what may in some instances be reasonably deduced in the absence of history.

Space-occupying lesions, particularly those which develop rapidly frequently produce vascular compressions with resultant swelling and hypoxia as occurred in the case now summarized.

Case 17. (51-287). This boy was 1 year old when he was thrown against a wall by his father. Upon arrival at the hospital a subdural hematoma was found over the left hemisphere. There was bilateral detachment of the retina. The subdural hematoma was

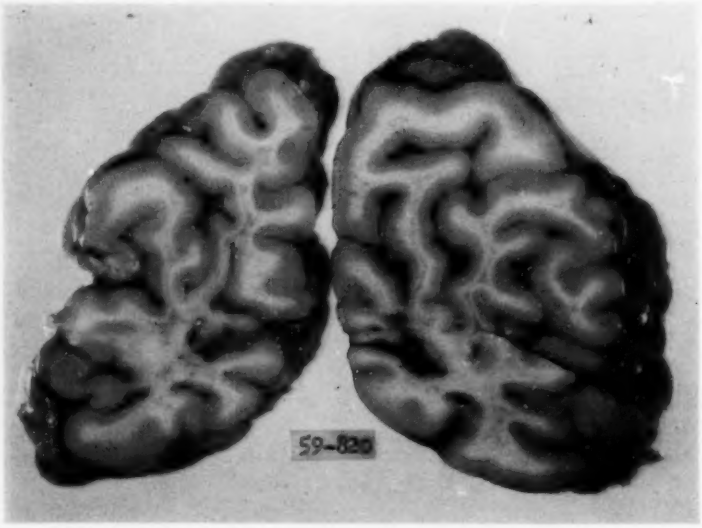


FIG. 20. P.B. (531-59; 59-820). The section shows sclerosis of the calcarine cortex bilaterally. The lesions were ancient. The 35-year-old man was dead on arrival at hospital after a motor car accident. Quite possibly the defective vision he must have had for years has something to do with the accident.

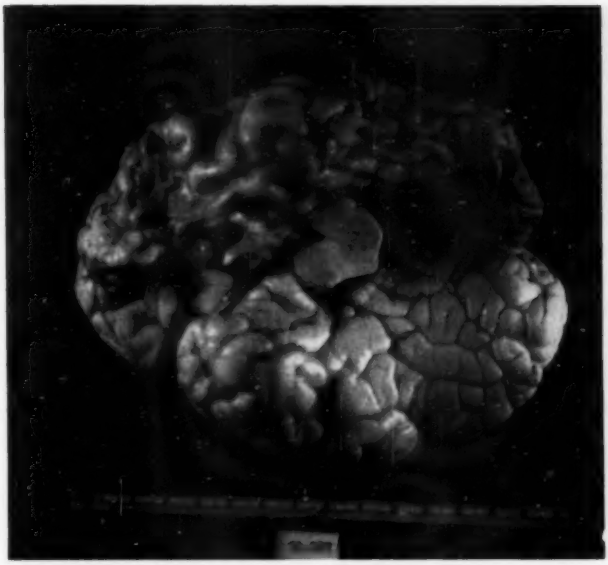


FIG. 21. J.W. (19-51; 51-287). There is severe atrophy of the left hemisphere particularly in the occipital and frontal lobes. The optic nerves did not show atrophy.

evacuated. The child remained paralyzed on the right side. He developed epileptic seizures, never talked and remained mentally retarded until death occurred about 1 year after the injury. The optic nerves were not atrophic.

Occasionally vast destruction of the cerebral hemispheres comparable to that seen with hydranencephaly may be observed (see Fig. 15) in children who were normal at birth. Probably the same mechanism, compression of the carotid arteries is responsible in both instances, before birth with hydranencephaly and soon after birth as in Case 18 now summarized.

Case 18. L.F. (236-60. 60-339), a 23-months-old Negro male died following aspiration of stomach contents. At birth he weighed 6 lb. 7 oz. The pregnancy and delivery were not remarkable. Growth and development were normal until the child was 3 months old. Then he developed wheezing respirations and vomiting and became unresponsive. When he was admitted to hospital the fontanels were bulging. There were foul-smelling excoriations on the neck. There was bilateral papilledema. The extremities were flaccid. He was comatose and responded only to pinprick. The spinal fluid was grossly bloody, pressure 220 mm; cultures on it were negative. X-rays showed separation of sutures.

One week later gastrointestinal bleeding developed and because of the resultant anemia he was given transfusions. In a further two weeks he was slightly improved but was rigid throughout and had non-reactive pupils. Two months after admission an EEG showed pronounced depression of cortical activity bilaterally. He had 3 convulsions during his stay in hospital. After discharge from hospital he was observed several times because of vomiting. Death occurred at home.

Comment Case 18 Figure 22. The similarity between hydranencephaly (Case 13, Fig. 15) and Case 18 Figure 22 above exemplifying what properly may be termed "acquired hydranencephaly" is striking. In more mature brains where the loss of hemispheres is as shown in Figure 22 there is persistence of connective tissue not present in true hydranencephaly. In such cases as this and hydranencephaly (Case 13) interference with carotid circulation bilaterally seems the only reasonable explanation. One of

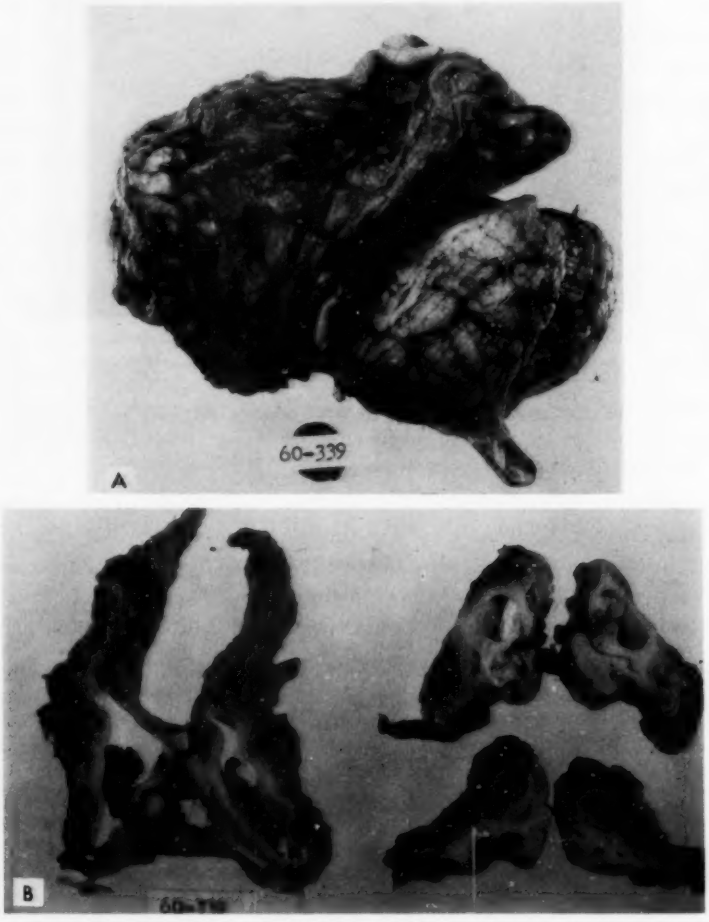


FIG. 22. L.F. (236-60; 60-339). A (*top*). The cerebellum is intact and a few convolutions of the occipital region remain. B (*bottom*). Sections through the parietal and occipital regions show severe loss of tissue. This is "acquired hydranencephaly". Compare with Figure 15.

us (R.L.) has seen such an autopsy finding as that shown in Figure 22 above to result from swelling of the brain in hemophilus influenza meningitis.

It is generally well known that brain damage persists after some inflammatory involvements. Residual damage may result from direct involvement of meningitis or meningoencephalitis. Also such damage may be due to vascular compression from brain swelling and associated hypoxia. In chronic meningitis, as for example tuberculous and syphilitic meningitis, endarteritis may produce areas of softening. Also meningitis may be complicated by venous thrombosis and dural sinus thrombosis when the resultant lesions will be situated according to the site of the thrombosis. Such thromboses may damage the visual pathways as is exemplified by the case summarized below.

Case 19. G.C. (797-58; 58-1188), a white boy, was 7 years old at the time of his death. At 1 year of age he suffered from influenzal meningitis. Thereafter he failed to develop, never walked or talked and was severely mentally defective. He was observed from time to time at the Baltimore City Hospitals, but after his original illness. As regards the eyes mention is made of intraocular hemorrhages having been observed on his first examination and of the right pupil being larger than the left.

Comment Case 19 Figure 23. The defects seen in A and B well may have resulted from bilateral obstruction to the veins draining into the sphenoparietal sinuses with the greatest damage affecting the left temporal lobe. It is obvious that the left optic radiation was destroyed entirely and at least Meyer's loop was destroyed on the right. Finally, as regards vascular involvements and hypoxia, even in early infancy an embolus may obstruct a cerebral artery. The resultant softenings may damage the optic pathways as is exemplified by Case 20.

Case 20. S.F. (53-29) was a 64-year-old man who suffered left-sided paralysis as a young child. No information is available as regards the onset of the paralysis. However, it was known that his eyes were thought to be normal externally, that the optic fundi were described as normal on ophthalmoscopic examination and that he had left homonymous hemianopsia. Death resulted from coronary thrombosis.

Comment Case 20 Figure 24. The smoothness of the walls of the cystic lesion signifies that the lesion had developed before the brain became mature. The intact ventricle wall suggests the lesion was softening rather than a massive hematoma. If this differentiation is correct the softening well may have resulted from arterial obstruction by embolism.

III. OPTIC ATROPHY IN INFANTS AND CHILDREN: THE ROLE OF HYPOXIA

Optic atrophy in infants and children frequently poses a problem in diagnosis. This is particularly true when tumor seems unlikely and when some obvious abnormality, such as for example oxycephaly or congenital

FIG. 23. G.C. (797-58; 58-1188). A (*top*). Partially cystic and partially sclerotic involvement accounts for almost complete loss of temporal lobes, more complete loss on left. B (*bottom*). Section at level red nucleus. Note almost complete absence left temporal lobe and severe scarring right temporal lobe.

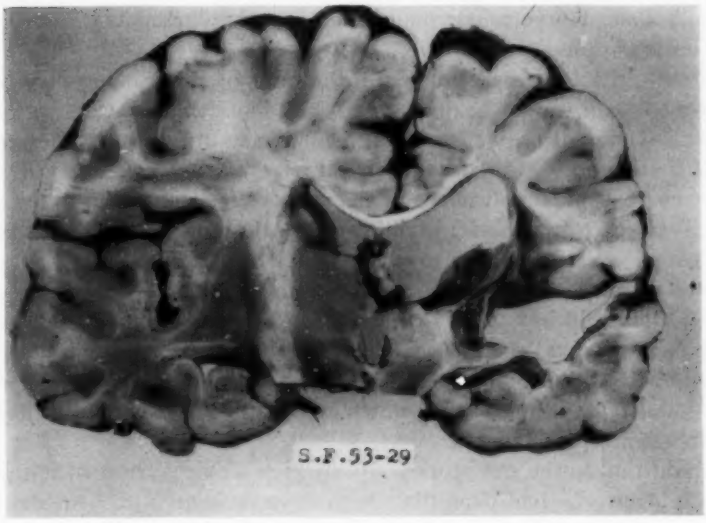


FIG. 24. J.N. (292-53; 53-29). The right hemisphere is small and there is pronounced hydrocephalus. The right thalamus and Ammon's horn are shrunken. The optic radiation on the right is interrupted. The lateral geniculate body is sclerosed.

hydrocephalus, does not exist. Maldevelopments of one or of both optic nerves in some instances are recognized with the ophthalmoscope, and such cases do not cause confusion. In some instances heredity is responsible as has been described by Kjer (20). We have observed several such cases, and certainly in some of them the optic atrophy has been present from birth or very early in life although we have not had an opportunity to make a really early diagnosis.

When optic atrophy occurs at an early age and an explanation for it is not available to the examiner the possibility of injury at birth is given consideration. If the optic atrophy is unilateral, and particularly if it is an isolated involvement, it has been generally believed that birth injury well may be the etiologic factor. The exact mechanism has not received any satisfactory explanation so far as we are aware. Birth injury which results in intracerebral hematoma is usually unilateral so that ipsilateral optic nerve atrophy might be the result of pressure. Bilateral optic atrophy usually is not attributed to birth injury. However, it has already been shown in this paper that hypoxia associated with swelling of both cerebral hemispheres does occur, also that both hemispheres may be involved by venous (dural sinus) compression. In such instances it might be anticipated that bilateral optic atrophy could result from pressure effects. With these ideas in mind it seemed reasonable that a study of case histories in the

Harriet Lane Home might establish some relationship between hypoxia and optic atrophy.

We selected 121 case histories on the basis of there being definite history of hypoxia at birth or during early childhood, and no case was studied when intracranial tumor was diagnosed. We found only 6 such cases. In all of these there was bilateral optic atrophy and in all of them there was other evidence of involvement of the nervous system. We have not as yet examined the case histories of children admitted to the Wilmer Institute as regards a relationship between hypoxia and optic atrophy.

Brief summaries of the cases of optic atrophy are now given. Certainly they do not help us as regards hypoxia and its possible importance as regards optic atrophy other than that severe hypoxia in some instances may be so associated. These case summaries indicate the difficulties surrounding our understanding the factors which result in optic atrophy in members of this age group.

In addition to the case studies from which Table VI was compiled several children who had been diagnosed as "anoxia at birth" were studied, and these studies are now summarized.

Case 1 of this paper exemplifies the uncertainties which exist as regards hypoxia at birth and cerebral development. In this infant what was considered as indicating severe central nervous system involvement provided a background for an entirely erroneous prognosis, for at 5 years of age the child was quite normal and unusually intelligent.

Case 21. E.P. (B67855), a Negro male infant weighed 3,207 grams at birth, breech delivery at home. It was uncertain as to how long the infant failed to breathe but not less than 15 minutes during which time it was observed by the ambulance attendant. There was extreme cyanosis when the hospital was reached. Breathing by intubation was instituted; manual assistance was necessary for 20 minutes. The infant exhibited fasciculations of the muscles of the face, arms, thighs. There was no cry or spontaneous movements of lower extremities. On the day after birth there was pronounced improvement. The cry was fairly normal. He moved all limbs and commenced sucking. Thereafter he improved rapidly. General physical examinations including examination of the eyes when the child was 10 months of age revealed no abnormalities.

Case 22. G.W.D. (R5786) a male infant weighed 8 lb. at birth born at 42 weeks gestation. Labor lasted 15 hours and delivery was by cesarian section. The umbilical cord prolapsed and the duration of prolapse was uncertain. There was difficulty with breathing (details not available) but oxygen was given during two weeks. There was no cry for several days also failure to suck. At 6 weeks the legs were crossed and rigid. There were frequent spells of stiffening when the head was turned to the right. The eyes rolled up during the spells which lasted for only a few seconds but occurred 20 to 30 times daily.

Our examinations were made when the child was 3½ years old. He was blind. The optic discs were extremely pale. The pupils did not react to light. When the head was manually turned to the left, the eyes turned upward to the right; when the head was turned to the right, the eyes moved upward. The head was constantly held extended on

TABLE VI

Hypoxia and Optic Atrophy

Name	Age Examined	Birth History and Hypoxia	Optic Fundi	Follow-up and Comment
R.L.G. A42854 Boy	11 weeks	3 weeks premature. Precipitous labor, rupture of cord. Considerable loss of blood. Required artificial respiration and oxygen, no cry for 3 days, hydrocephalus	Bilateral optic atrophy	Two years later, mentally retarded; delayed bone development
JA 531569 Boy	16 months	Dystocia Caesarian birth at term; delayed breathing immediately after birth. Convulsions at two months. Mentally retarded. Bilateral Babinski. Abnormal electroencephalogram	Bilateral optic atrophy. Spasmodic conjugate movements of eyes	
T.E.V. A80053	4 months	Normal birth at term. Some trouble breathing, but definite information not obtained. Polydactyly	Optic atrophy. Salt & pepper fundi	Cerebral malformation
M.M. A91800	22 months	Difficulty with breathing occurring several hours after birth. Pronounced mental defect. Electroencephalogram abnormal	Questionable optic atrophy, bilateral	
N.P. A21087	12 years	Difficult delivery at term, high forceps used. "Blue baby" at birth. Walked at 2 years, talked at 3 years, developed scoliosis at 7. Always had speech impediment. Pituitary dwarfism?, hypertension. Insensitivity to pain. Almost completely represented Riley-Day syndrome	Bilateral pale optic discs. "Dry eyes"	Probable malformation spinal and sympathetic ganglia
S.W. A40592 case record courtesy of Dr. F. R. Ford	After birth	Placenta previa in mother who had been exsanguinated. Pure nitrous oxide administered to mother for a few minutes by mistake. Apnea and severe cyanosis for 45 minutes. Artificial respiration and oxygen. Convulsions and rigidity for few days. Recurrent periods of apnea	Eyes deviated, jerky movements	At 8 months bilateral spasticity, increased tendon reflexes, small head, blindness, pale discs; minimal pupillary response to light. At 4 years blind, generalized rigidity, unable to talk or to sit or stand. Cortical atrophy. Enlarged ventricles

the neck. The neck was stiff; the legs were in a scissors position and they and the arms were stiff. The child seemed quite deaf. There was mild microcephaly. EEG's showed continuous seizures involving the entire brain, interpreted as a classical picture of myoclonic epilepsy.

Case 23. W.K.W. (R 5140) a male infant, 2 weeks premature, was born spontaneously, weight 5 lb. 8 oz. There was a definite history of placenta previa. Condition at birth was poor. There was a history of not breathing spontaneously for 30 minutes. He was given oxygen but there is no information as to how long. He had convulsions during the neonatal period. He received one blood transfusion. On discharge from hospital at 1 month he weighed less than 5 lbs.

At 10 years of age the child exhibited generalized choreo-athetosis. Development was retarded in all respects. The eyes were sunken. Lateral movements of the eyes to the left were obtained by rotating the head, but movements to the right could not be so obtained. Caloric stimulation failed to induce conjugate movements to the right. The pupils were normal and reacted to light. The optic fundi appeared normal. Visual acuity could not be estimated, but we thought he had some vision.

Case 24. D.R.D. (R.3959), a male infant, normal delivery, birth weight unavailable. The heart beat was not heard for 1 hour after birth. There was no information regarding breathing. There was severe jaundice in neonatal period. He was in hospital for 1 month.

At 18 years of age he exhibited spastic quadriplegia and severe mental retardation. The eyes were normal externally. Visual acuity could not be estimated but was present. The optic fundi seemed normal.

In addition to these five cases through the courtesy of Dr. Frederick Richardson and Dr. Stewart Wolff we have pertinent information concerning the ocular status of 45 children from the recently established Evaulation Center for Handicapped Children in Baltimore. More than 500 children have been examined. Dr. Wolff examined the eyes of ambulatory children in whom ocular abnormalities were suspected, and there were 11 of these. The optic fundi were normal in all. Visual acuity also was normal except when an eye was amblyopic on the basis of squint. Five children exhibited non-paralytic squint (esotropia 4; exotropia 1). Two children had epilepsy, 9 had hearing and/or speech defect, 4 were diagnosed as cerebral palsy. Comment. Part III of this paper concerning optic atrophy and hypoxia, presents insufficient evidence to justify conclusions. However this may be said as regards what has been stated.

Optic atrophy as reported in case histories studied and as observed in patients examined, was bilateral. It was associated invariably with other evidences of involvements of the central nervous system. Examination of Table VI indicates that optic atrophy may be associated with cerebral malformations when hypoxia has been recorded.

Examination of selected studies (Cases 1, 21, 22, 23, and 24) indicates the uncertainty which exists as regards residual cerebral involvements when there has been neonatal hypoxia. Ocular involvements other than optic atrophy occur in some such cases.

SUMMARY

The influences of hypoxia on the primary visual pathways of newborns and young children have been assessed on the basis of clinical and pathological studies. Certain correlations have been made and others suggested. We have considered hypoxia of systemic type such as occurs in asphyxia neonatorum, also hypoxia of localized type such as is produced by vascular compressions, venous thromboses or arterial obstructions. Birth injuries have been included because in many instances they are associated with localized hypoxia engendered by circulatory disorders.

In neonates, asphyxia or apnea neonatorum has been thought by some observers to be the most important factor in producing permanent brain damage. Our studies do not support this concept. Primary, or "uncomplicated", apnea within time limits as yet undefined, does not produce permanent morphologic brain damage in many instances, even when such disorders as convulsions and rigidity develop in the neonate. Such alarming occurrences may be purely functional and not associated with permanent brain damage.

On the basis of experimental work on animals a sudden transition from an adequate oxygen supply to severe hypoxia to anoxia produces extensive brain damage. Similarly it has been shown that a gradually increasing hypoxia merging into anoxia may not produce permanent cerebral changes. Our studies support the concept that since hypoxia is a normal state during the birth process an immediate subsequent period of anoxia (duration not established) such as occurs in asphyxia neonatorum, is infinitely less dangerous than a brief period of oxygen lack after adequate respirations had been established.

However, in what seems a significant minority of cases primary apnea neonatorum is associated with a symptomatology indicating permanent brain damage. In such instances areas of the brain stem and basal ganglia are involved so that the lesions are distributed as in kernicterus. Such seemingly selective involvements may be properly attributed to the difference in the maturity of the component parts of the brain. In the newborn infant the brain stem and basal ganglia are more mature than most of the cerebral cortex and the striate bodies. The greater maturity, and in consequence the difference in the rate of metabolism may serve to localize the pattern of permanent damage.

Swelling of the brain is a prominent feature observed at autopsy on newborns and young children. Whether the result of mechanical, thermal or chemical injuries or from toxic inflammatory states (when the damage is attributed to encephalities) swelling of the brain occurs in much greater degree than in adult brains.

On the basis of established knowledge concerning cardiac arrest and of

cerebral involvements associated with swelling of the brain and herniations of parts of it, observations at autopsy should explain much of what may result from hypoxia.

Significant dysfunction of the heart as a result of temporary circulatory stasis in the arterial border zones may produce cortical necroses involving both hemispheres symmetrically. In such instances since all major brain arteries terminate in the occipital region, the calcarine cortex and its vicinity are particularly liable to damage bilaterally, so that cortical blindness is produced, or incomplete damage results in localizing defects in the visual fields.

Also dysfunction of the heart is likely to produce brain swelling and edema and in consequence focal areas of necrosis from vascular compression. The visual pathways are particularly vulnerable on the basis of compression of the posterior cerebral arteries and their branches at the edge of the tentorium from the prolapsed hippocampal gyrus. Consequently the occipital portions of the visual pathways, including the calcarine areas, are liable to damage from localized as well as from generalized hypoxia. Compression of vascular supply in the region of the cornu ammonis and lateral geniculate body also exemplifies an influence of localized hypoxia.

Attention has been drawn to the probable importance of the particular type of heart failure as regards the disposition of cerebral lesions. If heart failure is predominantly left-sided the brain damage is more extensive in the gray matter, whereas if heart failure is predominantly right-sided (as for example in interstitial pneumonia) the white matter is principally involved. Obviously in many instances of heart failure such precise localization of lesions does not develop because with right-sided heart failure there is some concomitant failure of the left heart, and vice versa.

Asphyxia neonatorum may occur during the birth process as a result of prolapse of the cord. If not fatal, permanent brain damage when it occurs does not follow a definite pattern. In some instances rapid extraction and not prolapse of the cord may account for the damage.

Injuries of the brain resulting from deformation of the skull during birth have been extensively described. The primary lesions, most frequently an intracerebral hematoma involving one hemisphere may not directly involve the visual pathways. When secondarily involved the calcarine area or the lateral geniculate body, tract and nerve are affected ipsilaterally essentially in that order according to our observation of autopsy cases.

Attention has been drawn to massive involvements such as hydranencephaly. It seems probable that with cerebral edema and low blood pressure there is compression of both carotid arteries by the brain where they

enter the cranial cavity. If this occurs during intrauterine life, true hydranencephaly develops. If it occurs later the necrotic tissue in the supply area of the two arteries will not be completely resorbed as in the fetus, and thus what we have termed "false hydranencephaly" results. In all such cases of hydranencephaly brain swelling and compression of branches of the posterior cerebral arteries may account for damage to the visual cortices and to the lateral geniculate bodies and optic radiations.

To continue with vascular involvements, arterial embolism may occur in infants as a result of cardiac malformation or endocarditis. Depending upon the artery obstructed one or other portion of the primary visual pathways may be affected. The lesion is usually unilateral in contrast to bilateral lesions produced by heart failure. Also there may be unilateral or bilateral venous thromboses and sinus thromboses. Such lesions occur more often in children than in adults. Thrombosis of the superior longitudinal sinus may through pressure involve the optic radiations. Thrombosis of the sphenoparietal sinus may destroy the anterior half of the temporal lobe including fibers of Meyer's loop. Thrombosis of the great vein of Galen may cause necrosis in and around the lateral geniculate bodies and the calcarine area often bilaterally.

With a swollen brain pronounced swelling of the optic nerves and chiasm is observed frequently at autopsy. Traction displacement of the optic tract is seen often. What degree is compatible with return of function in infants and children who survive is not established.

Our clinical studies which indicate inadequacies of our present knowledge suggest generalizations.

(1) Apnea neonatorum in a great majority of instances does not have a harmful effect on cerebral development; such apnea we have termed "uncomplicated". It follows that other factors than apnea must be significant when cerebral defects persist. We believe that a period of apnea of sudden onset occurring after normal respirations have been established has dangerous possibilities as regards cerebral defects. We have suggested that cardiac deficiencies and shock may contribute to cerebral involvements in the neonate. Further knowledge in this area is essential.

(2) In cases included in this study, involvements of the primary visual pathways (when hypoxia either generalized or localized was a factor) were associated invariably with other involvements of the central nervous system. However, widespread cerebral involvements did not always include visual pathways involvement.

(3) Optic atrophy when present was bilateral in all the cases studied in this series.

(4) Although our studies indicated absence of primary visual pathways involvement as an isolated lesion and also absence of unilateral optic

atrophy (generalizations 2, 3), we are convinced that such involvements do occur but do so relatively infrequently. Furthermore, as regards optic atrophy, we have not presented here interesting but inconclusive evidence that herniation of the gyrus rectus, unilateral or bilateral, may be an etiologic factor.[2]

(5) Finally, it is again remarked that our observations and suggestions in this paper concern the primary visual pathways and not the higher pathways.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

November 14, 1960

MORPHINE TOLERANCE AND ADDICTION IN ANIMALS

E. W. Maynert and G. I. Klingman

In dogs, rats and rabbits, the acute administration of large doses of morphine sulfate (60–300 mg./kg.) caused less intense sedation than smaller doses (5–30 mg./kg.) but greater decreases in brain stem norepinephrine and adrenal epinephrine. Animals made tolerant to the sedative action of morphine by the subacute administration of drug had normal levels of brain and adrenal catechol amines. After the administration of large doses of morphine for 60 to 100 days brain and adrenal catechol amines were supranormal. The administration of a mono-amine oxidase inhibitor (1-phenyl-2-hydrazinopropane) caused a significantly greater increase in brain norepinephrine in morphine-tolerant than in control rats. The injection of nalorphine into morphine-tolerant dogs and rabbits produced excitatory "abstinence" syndromes accompanied by marked decreases in brain and adrenal catechol amines. Morphine-tolerant dogs anesthetized with pentobarbital prior to the injection of nalorphine failed to show changes in brain and adrenal catechol amines. In the rat the "abstinence" syndrome consisted of sedation and hypothermia and brain and adrenal catechol amines did not decrease.

In the light of evidence that catechol amines can stimulate the ascending reticular activating system of the brain stem, the liberation of these substances by morphine may be the cause of the decreased sedation after large doses given acutely. Likewise, adjustments in the synthesis, storage and release of catechol amines induced by the repeated administration of drug may explain the acquirement of tolerance to the sedative action of morphine. The failure of brain and adrenal catechol amines to decrease during "withdrawal" under anesthesia in dogs or during "abstinence" in rats suggests that the adjustments in the nervous system which constitute physical dependence on morphine do not occur in the adrenergic system and that this system is stimulated reflexly in an excitatory "abstinence" syndrome.

RECENT STUDIES ON A MECHANISM OF ACTION OF INSULIN[1]

Robert G. Langdon and Charles E. Mize

We have recently reported (1) that the isolated phenylalanyl chain of insulin qualitatively exerts the same effects as native insulin on the respiratory quotient (2) of adipose tissue *in vitro*; also, like insulin (3), this peptide promotes the incorporation of labelled glucose into fatty acids and respiratory CO_2. We now wish to report extensions of these observations.

By chromatography on ion exchange cellulose, we have purified glutathione reductase from rat liver 25,000-fold; the purified enzyme catalyzes the oxidation by GSSG[2] of 869

[1] This work was supported by a grant (H-1731(C6) from the United States Public Health Service.

[2] Abbreviations used are: GSSG and GSH are the oxidized and reduced forms of glutathione respectively. TPN and TPNH are respectively oxidized and reduced triphosphopyridine nucleotide.

TABLE I

Inhibition of Purified Hepatic Glutathione Reductase by the Phenylalanyl Chain of Insulin

Phenylalanyl Peptide	Moles Peptide/20,000 g. Enzyme	TPNH Oxidized	Inhibition
Molarity		*Millimicromoles min⁻¹*	*Per cent*
0	0	13.4	0
2.3×10^{-10}	0.1	13.4	0
1.1×10^{-9}	0.5	2.9	78
3.4×10^{-9}	1.6	1.4	90
3.3×10^{-8}	16.0	0.3	98
4.4×10^{-7}	210.0	0	100

TABLE II

Glutathione Reductase Activity in Tissues of Normal and Alloxan-Diabetic Rats

Tissue†	Hormonal Status	Glutathione Reductase Activity*	t	P
Muscle (28)	Normal	8.4 ± 2.7		
Muscle (28)	Diabetic	14.3 ± 6.2	4.77	<0.01
Liver (9)	Normal	78.8 ± 6.3		
Liver (8)	Diabetic	94.5 ± 5.3	5.55	<0.01

† The numbers in parentheses denote the number of animals in each group.

* Enzymatic activity is expressed as micromoles of substrate utilized per gram of protein per minute.

micromoles of TPNH per minute per mg. protein. It thus has a specific activity 24-fold larger than crystalline yeast glutathione reductase (4), and is approaching a homogeneous state. As measured by diffusion, the enzyme has a molecular weight of approximately 20,000. When incubated under strictly anaerobic conditions with stoichiometric quantities of the phenylalanyl chain of insulin, the purified enzyme is powerfully inhibited; representative data are given in Table I. It is important to note that the thiol functions of the phenylalanyl chain must be free in order to observe this inhibitory action; blocking these residues with mercaptide-forming reagents or oxidizing them to disulfide or higher oxidation states destroys this activity. Native insulin has no effect on the activity of this purified enzyme *in vitro*.

If it is presumed (1) that native insulin is a "prohormone" which is converted to its constituent chains at its site of action, and that one physiological action of the phenylalanyl chain is inhibition of glutathione reductase, one would predict that the activity of glutathione reductase would be elevated in the tissues of diabetic animals. Furthermore, it would be anticipated that insulin administration to intact normal or diabetic animals would lead to decreased glutathione reductase activity in tissues of the treated animals.

It is apparent from the data in Table II that the specific activity of glutathione reductase is indeed increased as predicted in soluble extracts of muscle and liver tissue from alloxan-diabetic rats.

To determine whether or not the administration of native insulin is followed by decreased activity of glutathione reductase in the tissues of intact animals, the following experiments were performed. A series of normal and alloxan-diabetic rats were lightly anesthetized with pentobarbital, and one epididymal fat pad was excised from each

animal. To one-half of the animals in each group five units of crystalline zinc insulin were administered by intramuscular injection, and their remaining epididymal fat pads were removed 45 minutes later. The control animals in each group were handled identically except that no insulin was administered. Each fat pad was homogenized and the soluble, high speed supernatant fraction from each preparation was assayed for glutathione reductase activity. It is apparent from the data in Figure 1 that administration

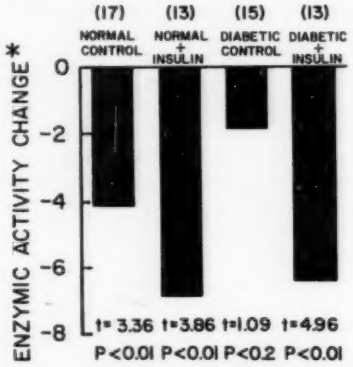

FIG. 1. Inhibition of adipose tissue glutathione reductase by administration of insulin to intact normal or diabetic animals.

* The results are expressed as the mean changes in glutathione reductase activity between the first and second fat pads of individual animals. The units of enzymic activity are micromoles of substrate utilized per gram of protein per minute.

of insulin resulted in significant decreases of glutathione reductase activity in adipose tissue from normal or diabetic animals.

These data further support the hypothesis that insulin may be a prohormone which is converted to its constituent peptide chains at its site of physiological action. It is suggested that one important action of the phenylalanyl chain is to inhibit glutathione reductase. These concepts are summarized in Figure 2.

$$\text{Insulin} \xrightarrow{\text{"Insulin reductase"}} \text{Phenylalanyl Peptide} + \text{Glycyl Peptide}$$

$$\text{Phenylalanyl Peptide} + \text{Glutathione Reductase} \longrightarrow \text{Enzymically Inactive Product}$$

$$\text{GSSG} + \text{TPNH} \overset{//}{\underset{//}{\rightleftharpoons}} 2\text{GSH} + \text{TPN}$$

FIG. 2. A possible mechanism for the action of insulin.

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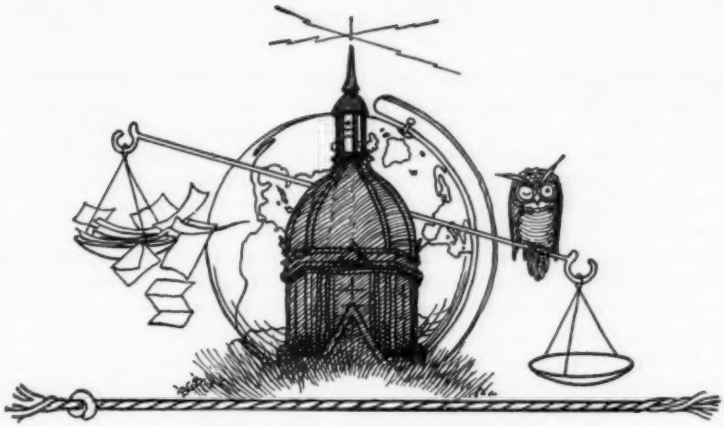

BOOK REVIEWS

Procedures in Vascular Surgery. By RICHARD WARREN. 211 pp., $12.00. Little, Brown & Co., Boston, Mass., 1960.

This is a well illustrated manual of procedures in vascular surgery as performed by the author, initially gotten together as an outline for house officers. The presentation is straightforward and well organized, but the same organization which makes the book attractive also requires the omission of controversial topics and applies a rigidity which is difficult to follow in surgical practice. These deficiencies are recognized with apologies by the author. There is also a recognized weakness in presenting only a single manner of performing a given task. The procedures of the author, however, by and large, are those which are widely accepted.

Although aims, indications, contraindications, and other topics are covered, there is perhaps too little "sermonizing," which the author hoped to avoid, and more explanation is needed to understand what is being attempted and why. The author assumes in his readers a certain knowledge of vascular surgery and proceeds to present in an orderly fashion his outline of care before, during, and after operation.

Particularly helpful are sections on ancillary measures in vascular surgery such as the nonoperative care of ischemic extremities, amputations, and the care of amputees, rehabilitation, etc. The nice coverage of these topics reflects the author's long-standing interest and excellence in the field rather than an entry into it recently when youth and aggressiveness have been the hallmarks of vascular surgeons.

The illustrations are good, the text well written. The wide margin allows one to annotate the book on the basis of his reading and experience. The book should be especially helpful to residents and house officers but will be of interest to others in the field of vascular surgery.

HENRY T. BAHNSON

Congenital Malformations. Ciba Foundation Symposium. Edited by G. E. W. WOLSTENHOLME and CECILIA M. O'CONNOR. 308 pp., $9.00. Little, Brown & Co., Boston, Mass., 1960.

This volume, the most recent of the Ciba Foundation's excellent series of publications, contains the text and discussions of thirteen papers presented at the Ciba Foundation Symposium on "Congenital Malformations" held in London in January, 1960,

under the chairmanship of Professor W. J. Hamilton of Charing Cross Hospital Medical School. The 29 participants include many internationally well known investigators and are drawn from a remarkable variety of disciplines. Active discussion is recorded between anatomists, clinicians, embryologists, geneticists, pathologists, pharmacologists, physiologists, psychiatrists, and veterinarians, and the resultant exchange of ideas makes stimulating reading and raises hope for rapid advances in this field in the future.

It would seem that the study of congenital malformations has at last proceeded beyond the descriptive stage and has advanced to the problem of how teratogenic agents produce their effects. Much of previous work on the incidence of congenital disease in various populations is reviewed in addition to the vast amount of experimental work on the teratogenic action of cancer inhibiting chemicals, hypervitaminosis A, actinomycin D, irradiation and pterylglutamic acid deficiency. The role of mild endocrinopathies in pregnancy as a factor in congenital malformation of the foetus is well treated. Anencephaly is much discussed but remains as great a puzzle as ever.

However, the most exciting development recorded in this small book comes from the geneticists, namely the recent demonstration that structural and numerical abnormalities of the chromosomes may lead to specific human congenital disease. The observation of an extra chromosome in mongolism (trisomy of chromosome number 21) is reviewed with the other known human chromosome anomalies by Dr. C. E. Ford. This discovery was evidently of such recent date, that one of the other contributors still discusses the significance of maternal influenza in the 2nd or 3rd month of pregnancy in the aetiology of mongolism. The discussion following this paper suggests lack of good evidence implicating uterine hypoxia and maternal influenza as important teratogenic factors.

The difficulty in distinguishing clearly between environmental and genetical causes of congenital malformation is stressed by Professor Penrose in a thoughtful article. He emphasizes that changed environment, besides affecting cell metabolism directly, may cause changed somatic heredity, and this is a factor to be considered in the interpretation of examples of apparent environmental causation of malformation. The study of the genetic aspects has been rather neglected in the past.

Clearly as a result of this symposium, there has been an important exchange of information between the various disciplines concerned with the study of malformations, particularly between the geneticists and the environmentalists. This record of their meeting makes good reading.

MALCOLM A. FERGUSON-SMITH

Adventure to Motherhood. By J. ALLAN OFFEN. 68 pp., $2.95. Audio Visual Education Co. of America. Distributed exclusively in the U. S. by Taplinger Publishing Co., New York, N. Y., 1960.

This little book has been prepared for the pregnant patient and is going to be available for general purchase in book stores, as well as in a special form sold directly to obstetricians along with a self-taking history form and some supplements concerning cesarean section, diabetes, and other complications of pregnancy, the entire unit to be used as a kit in the prenatal education of the patient.

The book traces in glorious color the life of Betsy, an attractive young lady, through her first pregnancy and delivery. Along with the color photographs showing that Betsy is enjoying every moment of her pregnancy is a text which discusses pregnancy and, subsequently, labor and delivery in terms which should be easily understood by any average patient. There is also a list of the advice and admonitions which most obstetri-

cians would consider sound and the usual danger signals in pregnancy are included. Controversial areas are not invaded, in order that the book may have the widest possible utilization, and the pictures of the actual delivery are in good taste.

Although justified by the type of format employed here, the price of this little volume may preclude its use in some practices.

DAVID L. SIMES

Staining Methods, Histologic and Histochemical. By J. F. A. MCMANUS and ROBERT W. MOWRY. 423 pp., $10.00. Paul B. Hoeber, Inc., Medical Division of Harper & Brothers, New York, N. Y., 1960.

This book will serve as a valuable introduction to histological techniques for the uninitiated. In trying to fulfill the stated aims of selecting methods that were effective, reliable and easily mastered, the authors have eliminated many good methods. It was apparent that histochemistry involving organic chemistry more complicated than that encountered in introductory college chemistry was avoided. The book, therefore, should make pleasant reading for those who wish an introduction to the field.

The sections of the book dealing with areas in which the reviewer was especially familiar were found to lack depth and to suffer from some inaccuracy. Although there are serious omissions in Chapter IV dealing with proteins and fats such as methods for protein-bound sulfhydryl groups, and for active carbonyl groups with the agent hydroxy naphthoic acid hydrazide, the section dealing with carbohydrates favorably reflects the author's special experience. More disappointing was the section on enzymes. Only alkaline phosphatase and esterase were adequately covered. The authors object to an excellent method for acid phosphatase developed in 1955 on the grounds that the substrate must be synthesized. Just about all substrates in enzyme histochemistry must be synthesized. They are apparently unaware that this substrate and several others are available from commercial sources. The unreliable method for phosphamidase would have been better omitted, and the Tween method for lipase (Gomori) is included as a method for lipase when it has been known for many years and acknowledged by the late Dr. Gomori himself that this method does not demonstrate lipase, but the ubiquitous esterase. There are much better methods for cytochrome oxidase than the one they have selected. The most reliable enzymatic histochemical method available today for demonstrating a host of dehydrogenasees with the use of the tetrazolium salt, Nitro-BT are not mentioned. The methods for the dehydrogenases that are given are out-dated and are only of historical interest.

Many will consider Chapter V the most valuable part of the book. In a novel arrangement, staining methods for special cells, tissues and organs are grouped by anatomic regions and here the senior author's large experience and pathologic know-how is revealed with greatest effect.

ARNOLD M. SELIGMAN

Cellular Aspects of Immunity. Ciba Foundation Symposium. Edited by G. E. W. WOLSTENHOLME and CECILIA M. O'CONNOR. 495 pp., $10.50. Little, Brown & Co., Boston, Mass., 1960.

In the last few years there has been increasing interest and investigation concerning the cellular aspects of immunity. Under the stimulation of work by Nobel Laureates Medawar and Burnet, more and more emphasis has been placed on the characteristics of the immunologically competent cell rather than the antibody. This symposium brings together 32 outstanding participants from various countries. The papers and informal

discussion that follow cover very well some of the most recent work and concepts in "cellular immunology." The volume considers the effects of various immunological processes on cell populations; the initiation, storage and transfer of immunological memory, the relation of humoral antibodies to cellular immunity and, finally, the situation of auto-antibody formation where the mechanism of "self recognition" presumably fails. A partial list of titles and authors are as follows: Identification of Immunologically Competent Cells by Simonsen; Theories of Immunological Tolerance by Medawar; Panel Discussion on the Clonal Selection Theory of Antibody Formation by Burnet and Lederberg; The Cellular Basis for the Immunological Memory by Dresser and Mitchison: Some Biological and Immunological Properties of Transfer Factor by Lawrence; and Interactions Between Sessile and Humoral Antibodies in Homograft Reactions by Gorer.

This book cannot be too highly recommended. It should have almost universal appeal to those interested in biology and medicine. The book has special value and significance for the immunologist, those internists concerned with allergy and infectious disease, "auto-immune diseases," and for the surgeon who is seriously interested in homotransplantation.

GEORGE W. SANTOS

Diseases of the Newborn. By ALEXANDER J. SCHAFFER. With a Section on Neonatal Cardiology by Milton Markowitz. Foreword by Harry H. Gordon. 878 pp., $20.00. W. B. Saunders Co., Philadelphia, Pa., 1960.

This extremely valuable book, as outlined in the author's preface, is a text devoted to the recognition, diagnosis, and management of diseases of the newborn infant during the first month of life. The prospectus of this book was to provide a reference text in which one could find comprehensive clinical descriptions of the disease states which occur in newborn infants. This purpose is richly fulfilled. The text is subdivided into sections dealing with the diseases involving individual organ systems of the newborn. Infections involving more than one organ system, jaundice in the newborn period, inborn errors of metabolism, and certain tumors are treated separately. The appendixes contain helpful information for resusitative procedures, drug dosage, and neonatal care.

The text contains actual case presentations to illustrate the diseases under discussion. The case abstracts are particularly helpful because the critical laboratory studies are included as well as reproductions of x-rays, autopsy material, and surgical specimens. Of particular importance, the case abstracts are easily read.

The discussions of signs and symptoms and the physical findings are presented with an authenticity which reflects the authors enormous experience and clinical acumen. The chapters dealing with the respiratory and cardiovascular diseases are more fully treated than the other diseases. This extended treatment is appropriate as well as fortunate, because these two organ systems are the ones most commonly involved in newborn diseases. The authors have integrated their own observations with the valuable contributions of others particularly well. The authors have deliberately restricted their discussions from excessive theoretical considerations which makes this book all the more reliable as a textbook. The bibliographies at the end of each chapter provide abundant source literature as well as current research developments. The cross-references are pertinent and the text has been well-indexed to make this book very useful. The only possible real lack in this text is the absence of any discussion in management of parenteral fluid therapy or disturbances in mineral metabolism.

Doctor Schaffer's book is of considerable value to anyone involved with the care of

newborn infants. His clinical descriptions of symptoms and findings on physical examinations of newborn diseases are not likely to become outdated.

G. B. ODELL

The Office Assistant in Medical Practice, 2nd edition. By PORTIA M. FREDERICK and CAROL TOWNER. 407 pp., $5.25. W. B. Saunders Co., Philadelphia, Pa., 1960.

In their preface, the authors state that "this book has been written to provide a general orientation to the whole field of assisting and to draw clearly the relationships of the various aspects of the job". They have succeeded admirably in achieving their objective and have produced a comprehensive but concise handbook which should be invaluable to novice and veteran in the field of medical office assistance.

Technical aspects of medical office assisting are described in detail, but the emphasis is on the importance of the individual patient.

ELEANOR FRY

Ocular Vertical Deviations and the Treatment of Nystagmus, 2nd edition. By J. RINGLAND ANDERSON. 189 pp., $8.50. J. B. Lippincott Co., Philadelphia, Pa., 1960.

This useful book is a valuable reference for any practicing ophthalmologist, who does occasional vertical muscle surgery. Of use principally as a reference, it has the great advantage of being concise. While it is comprehensive in this narrow field, there are many instances in which the reader will wish the author had extended his remarks to cover horizontal muscle imbalances as well. Remarks on the "A" and "V" syndromes are naturally limited, but are in keeping with current American thought on these two variations. One wonders why the treatment of nystagmus figures so large in the title, since only seven pages, liberally illustrated, are devoted to the subject, and the conclusion is that there are very few ways in which to treat nystagmus effectively.

I would especially recommend this book to the serious student of squint.

STEWART M. WOLFF

Surgery and Clinical Pathology in the Tropics. By CHARLES BOWESMAN. 1068 pp., $22.50. E. & S. Livingstone Ltd. Edinburgh and London, 1960. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

Mr. Bowesman's delightful book is based on twenty-five years' experience in medicine and surgery, mostly in West Africa. Throughout his career he maintained a "Medical Scrap Book" on the advice of one of his professors, in which he recorded notes of unusual cases and clinical oddities. From these notes he has written the present volume: "a series of essays on situations and subjects as they occur in clinical practice."

The volume is a remarkable compilation of descriptions of diseases peculiar to the Tropics, with extensive remarks on the medical and surgical measures employed by the author in the treatment thereof. Mr. Bowesman has not limited himself simply to surgery. Throughout much of his career he obviously had no one to rely upon for most of the hospital services we take for granted. Interspersed with descriptions of operative technique (most of which is excellent) the reader finds instructions on housekeeping, sterilization procedures, postoperative care etc. etc. etc. all adapted to situations in remote primitive regions. In addition, he has a facile pen and is a skilful observer. The chapter on "Ritual Operations in the Tropics" could well have been written by Margaret Mead and some of his passages are reminiscent of Isaac Dinesen. Further, he does not confine himself to any one field of surgery but also covers obstetrics, gynecology, opthalmology, urology and general tropical medicine.

In view of the present situation in West Africa this is a timely book, of great interest to almost any physician. It is highly recommended for inclusion in the library of any one intending to practice or study in the Tropics. For those of the rest of us who are occasionally encountering imported diseases of tropical origin it is a good reference manual to have available. This reviewer put aside his novels and read it many evenings simply for pleasure.

ALAN C. WOODS, JR.

Significant Trends in Medical Research. Ciba Foundation 10th Anniversary Symposium. Edited by G. E. W. WOLSTENHOLME, CECILIA M. O'CONNOR and MAEVE O'CONNOR. 356 pp., $9.50. Little, Brown & Co., Boston, Mass., 1960.

The idea behind this symposium was excellent: to assemble leaders in medical research from America, England, Germany, Sweden, Australia, Switzerland, South Africa, and elsewhere and have them indicate what they consider the most significant and promising areas of current work. The result makes interesting and worthwhile reading.

Specific areas discussed were molecular structure in relation to biological function and disease (Pauling), enzymology (Theorell), virus chemistry (Schramm), population dynamics of body cells (Burnett), medical genetics (Waldenstrom), neurophysiology (von Muralt), hormones (Young), the pancreas (Best), chronic pulmonary disease (Richards), malignant disease (Haddow), clinical nutrition (Brock), and hypertension (Pickering). But the formal presentations were merely points of departure for valuable discussion of what the most fruitful approaches to these several problems is likely to be.

Finally, Dr. James A. Shannon, director of the National Institutes of Health, spoke on administrative aspects of medical research. He stimulated valuable discussion, by representatives of many different countries, of how financial support is best provided for research and some of the various types of organization in which productive investigation can be conducted. For example, Linus Pauling made the following significant comment:

> Something has happened in some places, in fields such as chemistry, in the United States, that might be significant in rather a broad way. That is a change in the nature of professorships. This is perhaps best illustrated by the University of California at Berkeley ... There are now about 25 full professors of chemistry at Berkeley, and there are also several in biochemistry and other fields. These professors of chemistry are independent investigators—no one tells them what to do. But they do not have 25 institutes that each man has built up and runs for himself. There is one department of chemistry and there are facilities available for all of these men to work; this situation simplifies life a great deal for them. There are more professors of physical chemistry in the University of California at Berkeley than there are in the whole of France. This is the type of career that is best for a research man—to have a professorship that does not involve a heavy burden of administrative work or teaching, but makes available to him a supply of students who can learn from him, and provides materials of all kinds, which may be very expensive and could hardly be got for a one-professor institute ...
>
> ... They get students from a common pool selected by a committee that this group of professors appoint. When the student comes to the Institute, he himself makes the decision as to which of these professors he will work with.
>
> In many places this would be a satisfactory solution, but there are great difficulties in achieving it. It has been achieved in the United States because we have a complete lack of standardization in the institutions of higher learning. The directions in which these institutions move are determined by the people in them alone and not by a national decision (pp. 323–324).

VICTOR A. MCKUSICK

Glaucoma. Transactions of the Fourth Conference, March, 1959. Edited by FRANK W. NEWELL. 257 pp., $8.00. The Josiah Macy, Jr. Foundation Publications, New York. N. Y., 1960.

The purpose of this publication is to share as much as possible, the conference process with the absentee audience. This conference group represents one of twenty such sponsored by the Josiah Macy, Jr. Foundation and is an attempt to stimulate research and promote communication across departmental walls.

The participants are limited to 25 (members and guests) selected to represent a multidiscipline approach to an urgent problem in the field of medicine and health.

The contents of the conference are informal discussions by leading investigators in the ophthalmological field highlighting certain aspects of their research punctuated by lively questions, comments and criticisms from other members and guests.

This volume includes information dealing with trabecular structure and its probable role in simple glaucoma in man; histology and structure of ciliary epithelium and its relationship to aqueous secretion; and the histologic and pathologic findings in six eyes with primary simple glaucoma.

The participants are:

(1) Levon K. Garron of the Proctor Foundation for Research in Ophthalmology, University of California Medical Center. His presentation is concerned with the electron microscopic appearance of the trabecular meshwork in human eyes. This area is probably involved in glaucoma.

(2) Abe S. Holmberg of the Department of Ophthalmology, Washington University School of Medicine. He deals first with the inner wall of Schlemm's canal and the ultrastructure of the normal outermost layer of the trabecular apparatus in man. Later he discusses the fine structure of the ciliary epithelium and its relationship to aqueous secretion.

(3) Norman Ashton from the Department of Pathology, Institute of Ophthalmology, University of London.

His discussion revolved around the significance of mucopolysaccharide in normal trabecular meshwork and pathological findings in the outflow pathways.

(4) George D. Pappas of the Department of Anatomy and Ophthalmology, Columbia University, College of Physicians and Surgeons. He inspires and directs discussion and study of the ultrastructure of the ciliary epithelium and its relationship to aqueous secretion.

(5) Abraham L. Komzweig of the Department of Ophthalmology, Mt. Sinai Hospital, New York. He illustrates and discusses the pathological findings in six eyes with primary glaucoma.

Among the distinguished guests who participated in this conference were, Dr. A. Edward Maumanee, Professor of Ophthalmology of this University and Dr. Bernard Becker, a former resident at Wilmer and now Professor of Ophthalmology, Washington University School of Medicine.

There is no summary but the text is well illustrated and has a comprehensive reference list at the end of each discussion topic. It is enthusiastically recommended for the research and pathology student.

RICHARD E. HOOVER

BOOKS RECEIVED FOR REVIEW

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Clinical Orthopaedics, No. 17. Clinical Physiology and Pathology of Bone. Edited by ANTHONY F. DE PALMA. Guest Editor, MARSHALL R. URIST. 387 pp., $7.00 single number. J. B. Lippincott Co., Philadelphia, Pa., 1960.

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The Management of Fractures and Soft Tissue Injuries. By the Committee on Trauma, American College of Surgeons. Based on An Outline of the Treatment of Fractures, 7th edition and Early Care of Acute Soft Tissue Injuries, 2nd edition. 372 pp., $5.00. W. B. Saunders Co., Philadelphia, Pa., 1960.

Medicine as an Art and a Science. By A. E. CLARK-KENNEDY AND C. W. BARTLEY, 425 pp., $6.25. J. B. Lippincott Co., Philadelphia, Pa., 1960.

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Rypins' Medical Licensure Examinations. Topical Summaries and Questions, 9th edition. Edited by WALTER L. BIERING. 805 pp., $11.00. J. B. Lippincott Co., Philadelphia, Pa., 1960.

Surgical Diseases of the Pancreas. By JOHN M. HOWARD AND GEORGE L. JORDAN. 607 pp., $20.00. J. B. Lippincott Co., Philadelphia, Pa., 1960.

A System of Medical Hypnosis. By AINSLIE MEARES. 484 pp. $10.00. W. B. Saunders Co., Philadelphia, Pa., 1960.

OSTEOCHONDROMA INVOLVING THE FIRST RIB WITH NEUROVASCULAR COMPRESSION

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Osteochondromas are the most common of the benign bone tumors. They comprise about 30 per cent of Geschickter and Copeland's (1) series of osseous neoplasms, and approximately 50 per cent of the series described by Jaffe (2). By far the commonest sites of occurrence are the distal end of the femur and the proximal ends of the femur and humerus. It is quite rare for them to occur on the ribs. Jaffe reported none, while Geschickter and Copeland reported one in 310 instances. Dahlin (3), who had the largest collection occurring on the ribs, reported nine cases in 272 tumors. The ribs involved were not enumerated. According to Dahlin, an osteochondroma is the most common benign bone tumor affecting the ribs.

While many of these tumors escape clinical observation because of the absence of symptoms, the majority give evidence of their presence by a painless swelling of the bone or by stiffness with "rheumatic" pains in the neighboring joint. The average duration of these symptoms is about five years before a definitive diagnosis is made. We would like to present a case of an osteochondroma involving the first rib, producing the chief complaint of pain and swelling of the right arm.

Case E.E. #208441. This 32-year old white female was first seen at the Guthrie Clinic on February 18, 1960 with the chief complaint of pain and swelling of the right arm of many years duration, dating back to childhood. About the age of 16 years she remembers having had an episode of considerable discoloration and marked swelling of the right arm. Such episodes have recurred intermittently until the present time, becoming progressively worse in the past few years. The pain is described as a dull aching sensation mainly on the ulnar side of the right forearm and hand. On moving her right shoulder she has noted a crunching and grating sensation under the clavicle, and on rotating the right arm and shoulder backwards she has noted a fullness just under the clavicle.

Examination of the patient revealed tenderness in the right upper clavicular area when the head was rotated to the left, but there was no limitation of motion. There was no weakness or atrophy of the neck muscles. The left shoulder was entirely normal. Examination of the right shoulder revealed a fullness beneath the mid-portion of the clavicle. When the shoulder was rotated posteriorly the examiner could feel crepitation in the mid-clavicular region. A mass was also palpable beneath the clavicle measuring approximately 2 × 4 cm., firm in texture and non-tender. It was not attached to the

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clavicle but was freely movable under it. It seemed attached to the underlying structures. The right upper arm was a half-inch larger than the left and the right forearm was also a half-inch larger than the left. No definite weakness of the right arm could be elicited. The right biceps tendon reflex was hypoactive but all other reflexes were physiologic. The Allen and Adson tests for radial pulse obliteration were negative. The skin of the right arm was slightly cyanotic but showed no other changes.

Roentgenogram of the right shoulder (see Fig. 1) revealed a dense, calcified mass arising from the right first rib at the junction of the anterior and middle third, projecting upwards just behind the clavicle. A diagnosis of osteochrondroma or chondroma of the first rib was made. The patient was advised to have this mass removed but she demurred. Because of increase in pain she returned one month later for excision of the tumor. The findings on admission to the hospital were essentially the same as those one month previously except that there was no measurable difference in the circumference

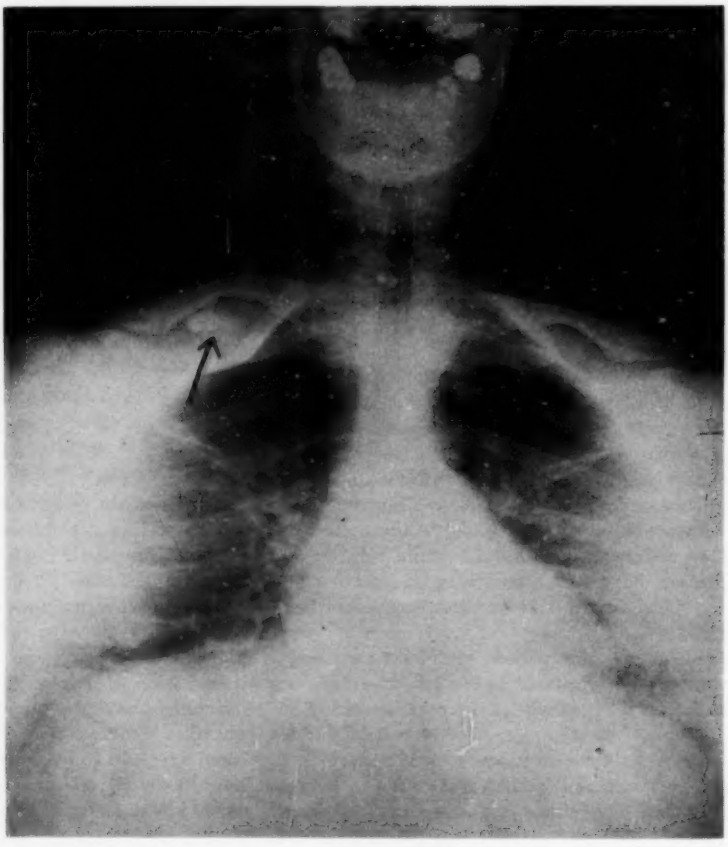

FIG. 1. X-ray of chest showing mass under right clavicle, on first rib. Mass could be palpated in the neck, above the clavicle.

of the arms and she now had slight hypesthesia on the ulnar aspect of the right forearm and hand. The preoperative diagnosis was that of an osteochondroma of the right first rib producing neurovascular compression.

OPERATION: With the patient in a supine position and the right arm abducted and extended, an incision about 20 cm. long was made parallel to the sternum, extending from the right supraclavicular fossa downward over the clavicle, and curving slightly towards the right breast. The skin and subcutaneous tissues were dissected away from the clavicle and pectoralis muscles. The pectoralis muscles were cut away from their origin, stripped off the clavicle and chest wall, and retracted laterally. The tumor was now easily palpable just beneath the clavicle, covered by some loose fatty tissue. On further dissection, a bursa was encountered at the top of the tumor which had formed between it and the clavicle. The tumor was seen to be arising from the first rib. The subclavius muscle was stripped from the inferior portion of the clavicle, as well as the muscles about the first rib and the parietal pleura on its under surface. The subclavian artery and vein and the lower cord of the brachial plexus were gently dissected from the superior portion of the tumor. With a Gigli saw the distal portion of the first rib was cut near its sternal attachment. The rib and tumor were freed up and its proximal portion was cut with bone shears. The subclavian artery was seen to fall into the place occupied by the tumor. The cut ends of the rib were trimmed and waxed. The tumor was removed *in toto* with the section of rib attached to it. The wound was closed in layers with interrupted silk sutures.

The pathological report was a benign osteochondroma (Fig. 2). Her postoperative course was uneventful and within a few days the swelling of the right arm had disap-

FIG. 2. Photomicrograph (low power) of neoplasm, showing typical structure of benign osteochondroma.

peared. By the third postoperative day the painful sensations in the arm had also disappeared. By the time of discharge she had good range of motion in the right shoulder but had some tenderness and tightness about the incision. She was seen for postoperative checkup six weeks after discharge. At this time she had full range of motion of the right shoulder and no muscle weakness was elicited. Deep tendon reflexes were all active and equal. There was no sensory loss and no swelling of the arm. She had had no recurrence of the pain or periods of cyanosis. At times she would experience some tightness about the incision but this was very mild.

COMMENT

According to Lichtenstein (4), osteochondromas appear to have their origin in perverted activity of the periosteum which tends to form anomalous foci of metablastic cartilage. The cartilage foci, by continued growth and enchondral ossification, may give rise to manifest exostoses. The sex incidence is about equally divided between males and females, and usually occurs in persons between the ages of 10 and 25 years. The vast majority of these lesions are benign and operative intervention is not called for, since in the usual exostosis the periosteum and the underlying cortical bone succeed eventually in "hemming" in the cartilaginous cap except at the point where the fibrous portions of the tendon perform an identical function. However, operative removal may become necessary because of the interference by the tumor with the proper function of the surrounding muscles, activity of an adjacent joint or, as in our patient, when it intermittently compressed the subclavian artery and vein, as well as irritating the lower cord of the brachial plexus.

According to Jaffe, only about 1 per cent of these tumors show malignant changes as indicated by rapid growth after years of quiescence.

SUMMARY

An osteochondroma arising from the first rib and producing neurovascular compression is presented. The operative approach for the removal of the tumor is described. This is a most unusual site and the final result was most satisfactory for this neoplasm.

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CLINICAL AND EXPERIMENTAL EVALUATION OF THE TRIIODOTHYRONINE (T3) RED CELL UPTAKE TEST

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Measurement of the red blood cell uptake of L-triiodothyronine *in vitro* has shown promise as a diagnostic test of thyroid function (1 to 6). However, the exact status that this test will achieve in clinical medicine remains uncertain. The purpose of this paper is to present the results of further clinical experience with the test and experimental studies designed to evaluate it.

Outline of the Development of the T3 Red Cell Uptake Test

In 1941 Riggs and his associates found the serum iodine level to be increased in untreated hyperthyroid patients (7). Further investigations indicated that the iodine was chiefly protein-bound (8), and that thyroxine iodine constituted approximately 80 per cent of the total protein-bound iodine (9). In 1952, three groups of investigators, utilizing paper electrophoresis, localized the primary thyroxine binding site to the inter-alpha globulin zone of plasma protein (10 to 12). In 1955, Albright, Larson and Deiss demonstrated that this thyroxine binding globulin (TBG) was relatively saturated with endogenous thyroxine in hyperthyroid patients and unsaturated in hypothyroid patients (13).

Experiments begun in 1954 by Hamolsky led to the development of a simple method for estimation of the degree of saturation of TBG in patients with thyroid diseases (1). Initially, Hamolsky observed that after I-131 labelled thyroxine was incubated in plasma from hyperthyroid patients and injected into dogs, the rate of clearance of radioactivity from the dogs' plasma was faster than when the preliminary incubation had been in plasma of euthyroid patients. Subsequently, he observed that the I-131 labelled L-thyroxine incubated in plasma from hyperthyroid patients had a faster rate of incorporation into rat diaphragm than when incubated in plasma from euthyroid persons. Erythrocytes as well as rat diaphragm could be used to measure these differences between hyperthyroid and hypothyroid patients, provided one used triiodothyronine rather than thyroxine. The work of Crispell and associates clarified the mechanism of this phenomenon (16). Erythrocyte binding of T3 in Hamolsky's procedure was postulated to be related chiefly to the degree of thyroxine saturation of TBG. Measurement of the triiodothyronine (T3) red cell uptake has subsequently been widely adopted as a test of thyroid function.

MATERIALS AND METHODS

Venous blood was withdrawn into syringes containing heparin. Hematocrits were measured by the micro-capillary method. Duplicate 3 ml. samples of whole blood were pipetted into 10 ml. Erlenmeyer flasks and I-131 labelled L-triiodothyronine (T3), 120×10^{-4} microgm.,[1] was added. The stoppered flasks were incubated for two hours at 37°C. Adequate mixing was insured by intermittent agitation of the flasks. Following incubation, the radioactivity of 1 ml. aliquots of whole blood was measured in a 3 inch well scintillation detector. The erythrocytes were then washed five times with 10 ml. volumes of 0.16 M sodium chloride, and the radioactivity remaining with the erythrocytes was determined. The percentage of triiodothyronine taken up by the red cells was calculated as follows:

$$\text{Per cent T3 Red Cell Uptake} = \frac{\text{net counts of washed red blood cells} \times 100}{\text{net counts of whole blood} \times \text{decimal hematocrit}}$$

Hematocrits below 30 per cent or greater than 50 per cent were adjusted to approximately 40 per cent by removing red cells or plasma prior to the test procedure. The reproducibility of the method was shown by the finding that the mean standard deviation of quadruplicate determination in 176 consecutive patients in the present study was 0.5 per cent.

The relationship between the plasma thyroxine content and the T3 red cell uptake was studied as follows: A stable stock solution of thyroxine was prepared daily by dissolving 4 mg. of powdered sodium L-thyroxine (T4)[2] in 1.3 ml. of 0.10 M sodium hydroxide which was diluted to a final volume of 10 ml. with 0.16 M sodium chloride. From this solution, thyroxine was added to plasma to give final concentrations of 0.1 to 0.8 microgm. per ml. After the plasma samples had been incubated for one hour at 37°C., washed erythrocytes were added to yield a final hematocrit of approximately 40 per cent. The T3 red cell uptake was then measured as previously described.

The effects of carbon dioxide and the metabolic inhibitors, 0.005 M sodium arsenate (14) and 10 mg. per ml. N-ethyl maleimide (15), on the T3 red blood cell uptake, were studied in a Warburg apparatus using Krebs Ringer bicarbonate buffer.

The patients' diagnoses were confirmed by evaluation of clinical and laboratory studies exclusive of the T3 red cell uptake test.

RESULTS

I. Role of Erythrocytes.

To study the binding of L-triiodothyronine (T3) and L-thyroxine (T4) by erythrocytes in the absence of serum proteins, washed erythrocytes were

[1] Obtained from Abbott Laboratories. Stock solutions of radioactive triiodothyronine were kept refrigerated at 4°C. and were discarded after one week.

[2] Obtained through the courtesy of Smith, Kline & French Laboratories.

added to Krebs Ringer bicarbonate buffer to yield a 40 per cent suspension. I-131 labelled T3 or T4 was added. After 30 minutes' incubation at 37°C., the amount of radioactivity on the red cells was measured after serial washings with 10 ml. of 0.16 M NaCl. At the concentration of T3 studied (120×10^{-4} microgm. per ml.), 83 per cent (range: 78 to 88%) of the added T3 remained bound to the red cells after the first 10 ml. washing. This decreased progressively to 65 per cent after four washings. When the suspended red cells were incubated with T4, instead of T3, (120×10^{-4} microgm. per ml.) 62 per cent (range: 58 to 67%) of the added T4 was bound to erythrocytes after a 10 ml. washing. These results indicated that the red cell uptake could not be explained on the basis of simple diffusion of T3 or T4 from plasma to red cell water.

To determine whether the uptake of these thyroid hormones by erythrocytes was an active metabolic process, the erythrocytes were exposed to the metabolic inhibitors sodium arsenate and N-ethyl-maleimide before adding T3 or T4. Although manometric measurements of carbon dioxide production indicated that red cell metabolism was markedly impaired by these substances, there was no significant decrease in the amount of either triiodothyronine or thyroxine bound to the erythrocytes. Thus, the red cell uptake of T3 and T4 is neither the result of simple diffusion from plasma to the red blood cells nor of an active metabolic process, but presumably is a physico-chemical binding phenomenon.

II. Relationship Between Plasma Thyroxine Level and T3 Red Cell Uptake.

Plasma thyroxine concentration was increased by adding L-thyroxine (0.1 to 0.8 microgm. per ml.) to plasma and incubating it at 37°C. for one hour. After incubation, O negative erythrocytes, previously washed in saline, were added to the plasma to make a 40 per cent suspension. I-131 labelled triiodothyronine (120×10^{-4} microgm. per ml.) was added and the T3 red cell uptake was measured by the previously described method. As shown in Figure 1, the red cell uptake was directly related to the amount of thyroxine in the plasma.

III. Factors Influencing the T3 Red Cell Uptake.

By utilizing this standardized system in which the plasma thyroxine level was varied, it was possible to study factors influencing the T3 red cell uptake.

(1) Effect of Carbon Dioxide.

High concentrations of carbon dioxide (100% carbon dioxide atmosphere) increased the red cell binding (Fig. 2). This effect of carbon dioxide

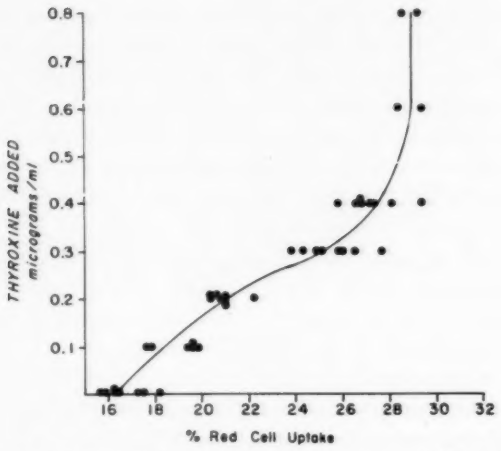

FIG. 1. Relationship between plasma thyroxine content and T3 red cell uptake.

provides a possible explanation of the increased T3 red cell uptake observed in patients with pulmonary ventilatory disease. On the other hand, at low partial pressures of carbon dioxide (produced by allowing blood to equilibrate with room air), red cell binding was markedly reduced below control values (Fig. 2). These results support the concept that it is mandatory to keep the blood in stoppered containers to prevent diffusion of carbon dioxide from the system prior to and during the test procedure.

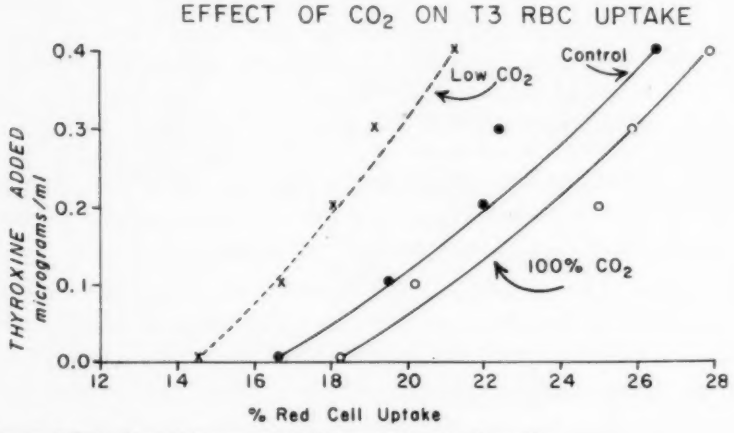

FIG. 2. Effect of partial pressure of CO_2 on T3 red cell uptake.

(2) *Effect of Inorganic Iodide.*

The presence of iodide at a concentration of 7 microgm. per ml. (at least 1,000 times normal iodide levels) did not greatly alter the T3 red cell uptake (Fig. 3) over the range of plasma thyroxine studied.

(3) *Effect of An Iodinated Radiographic Contrast Medium.*

The addition of the radiographic contrast medium, Hypaque(R) commonly used in intravenous urography, at a concentration of 10 microgm. per ml. of plasma, did not significantly reduce the triiodothyronine red cell uptake when studied at various thyroxine levels.

(4) *Effect of Thiouracil.*

The effect of thiouracil on the T3 red cell uptake is shown in Figure 4. Five micrograms of thiouracil was incubated with plasma at 4°C. for 18 hours. Thyroxine (0.1 to 0.4 microgm. per ml.) was then added; washed erythrocytes were added and the T3 red cell uptake performed. No effect of thiouracil was observed.

IV. T3 Red Cell Uptake in Thyroid Disease.

Figure 5 A, B summarizes the results of the T3 red cell uptake in patients with the clinical diagnosis of hyperthyroidism or hypothyroidism, compared to a group of euthyroid patients. Two of twenty-one female patients with hyperthyroidism had values within the euthyroid group. One

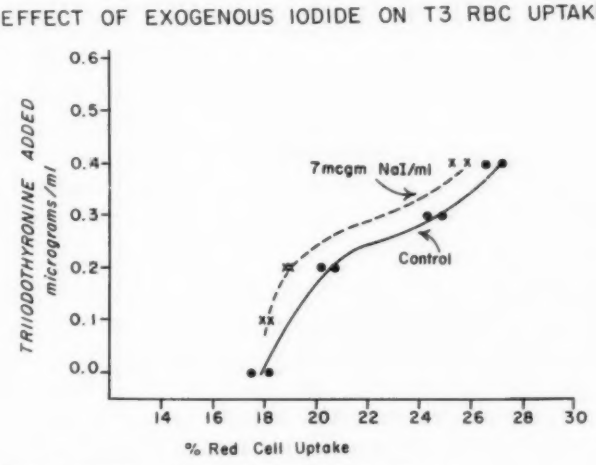

FIG. 3. Effect of exogenous iodide on T3 red cell uptake.

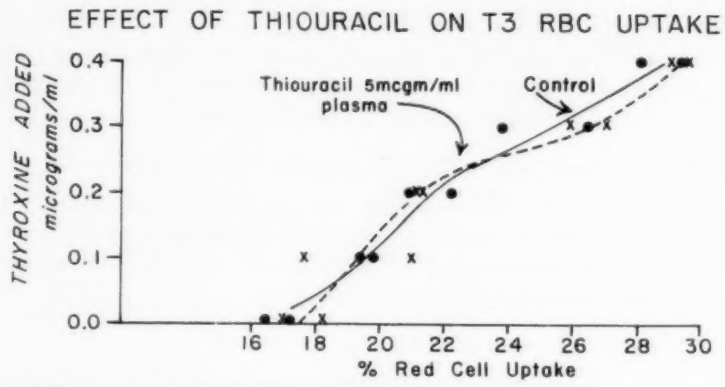

FIG. 4. Effect of thiouracil on T3 red cell uptake.

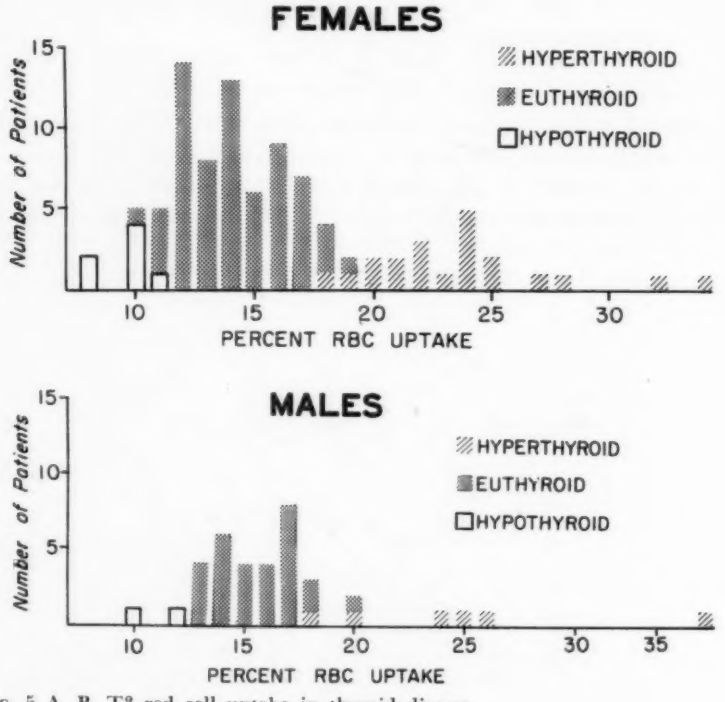

FIG. 5 A, B. T3 red cell uptake in thyroid disease.

TABLE I

Summary of Results of T3 Red Cell Uptake Determinations

Clinical Diagnosis	Sex	Number of Patients	Mean RBC Uptake	Standard Deviation
Euthyroid	Male	29	15.5	1.9
	Female	67	14.1	2.0
Total		96	14.8	2.0
Hyperthyroid	Male	6	24.8	6.3
	Female	21	23.8	3.8
Total		27	24.3	5.1
Hypothyroid	Male	2	10.9	
	Female	7	9.5	1.3
Total		9	10.2	
Euthyroid on Thyroid Therapy		1	16.6	
Hyperthyroid on Antithyroid Drug		3	15.9	
Hypothyroid on Therapy		4	17.6	2.0
Acute Thyroiditis		2	25.2	
Nephrotic Syndrome		4	23.4	4.8
Hypoalbuminemia (2.5 mg.% or less)		7	23.7	4.7
Chronic Lung Disease		3	22.6	
Testosterone Therapy		1	22.6	
Estrogen Therapy		2	10.0	
Anticoagulant Therapy		3	24.5	
Liver Metastases		2	20.8	
Uremia (Arteriosclerotic Renal Disease)		3	27.5	
Uremia (Gout)		1	17.0	
Paroxysmal Auricular Fibrillation		1	20.7	

of six male thyrotoxic patients had a value less than 20 per cent. Of the nine hypothyroid patients, at least two were within the euthyroid range. None of the hypothyroid patients had values lower than eight per cent.

Table I lists the mean values and standard deviations of the results obtained in hyperthyroid, hypothyroid and euthyroid patients, together with the values obtained in patients with other diseases.

V. T3 Red Cell Uptake in Diseases Unrelated to the Thyroid.

As shown in Table I, numerous diseases were associated with an increased T3 red cell uptake. At least two patients with each of the following diseases had significantly elevated values: nephrotic syndrome, chronic pulmonary ventilatory disease, liver metastases, uremia secondary to arteriosclerotic renal disease, and anticoagulant therapy.

Five patients with hypoalbuminemia secondary to various diseases (including two patients with nephrosis) had red cell uptake values ranging

from 21 to 30 per cent. To determine whether this was an effect of hypoalbuminemia *per se*, the albumin content of plasma from these patients was increased by adding 2.5 mg. of human serum albumin per 100 ml. of plasma. The T3 red cell uptake was then determined. Similar studies were performed in which 5.0 gm. per cent serum albumin was added. In no case was the T3 red cell uptake significantly lowered by correcting the hypoalbuminemia.

DISCUSSION

Initial studies were designed to clarify the mechanism of the T3 red cell uptake test. The present data, as well as those of others (16), are consistent with the mechanism proposed in Figure 6. In the test procedure a constant amount of T3 is added to whole blood. The added T3 equilibrates among three states: bound to plasma protein, bound to red cells, or free. In a situation in which no plasma proteins are present, after serial washings 65 per cent of the added T3 will be bound to red cells and the remainder will be free. This same fraction is bound even after the red cells have been metabolically inhibited by arsenate or N-ethylmaleimide, indicating that the role of the red cell is a secondary one in the test procedure and is a measure of the free T3 present. In whole blood of euthyroid patients, the added T3 is bound primarily, to plasma protein chiefly thyroxine-binding globulin. The red cells bind approximately 15 per cent of the added T3. Since thyroxine and T3 are both bound by thyroxine binding protein, the amount of added T3 that will be protein bound is determined by the number of binding sites occupied by thyroxine. In hyperthyroid patients, the protein-binding sites are occupied as a result of the high plasma thyroxine

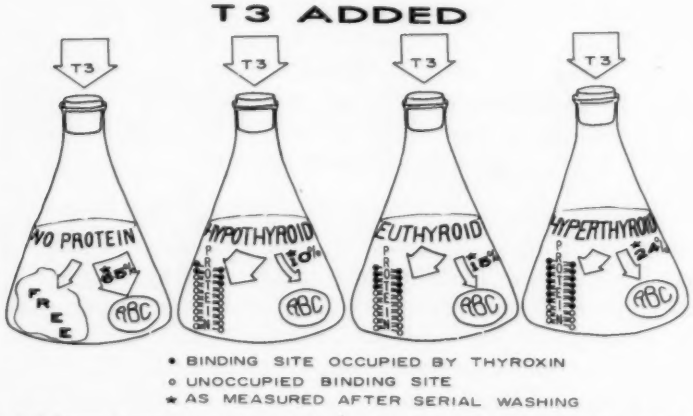

FIG. 6. Schematic representation of the mechanism of the T3 red cell uptake test.

level. Consequently, less of the added T3 is bound. Therefore the amount of T3 available for red cell binding is increased. In hypothyroidism, the number of binding sites occupied by thyroxine is decreased and less of the added T3 is bound to the red blood cells, since more is bound by thyroxine binding protein. Consistent with this hypothesis is the demonstration that the T3 red cell uptake is a function of the amount of T4 in the plasma (Fig. 1).

The T3 red cell uptake is usually elevated in patients with thyrotoxicosis. In patients with hypothyroidism the overlap into the normal group was significant. Therefore the test seems more valuable as a measure of increased rather than decreased thyroid function.

In clinical conditions not associated with increased thyroxine production by the thyroid, the T3 red cell uptake may also be increased. High values are found in patients with chronic pulmonary ventilatory insufficiency, nephrotic syndrome, and metastatic carcinoma involving the liver. This lack of specificity is the principal fault of the test, as a diagnostic procedure. On the other hand, the elevated T3 uptake in these conditions may prove to be of interest. The red cell uptake may be increased by two mechanisms, by an increased amount of endogenous or exogenous thyroxine in the plasma, or by a decrease in the number of thyroid hormone binding sites. A possible explanation for the elevation in these nonthyroid diseases is a decrease in the number of available thyroid hormone binding sites, either from a decreased amount of thyroxine-binding globulin or a competitive inhibition of the binding mechanism. Further investigations of these patients to determine the mechanism of the increased uptake are in progress.

The most significant advantage of the T3 red cell uptake test is that it can be used to demonstrate increased thyroid function in the presence of iodine-containing substances that invalidate the protein-bound iodine and radioiodine uptake tests. Twenty per cent of the 176 consecutive patients referred to this laboratory for diagnostic testing of thyroid function had a radiographic examination employing an iodine-containing contrast material within a few days or a week of the test. In these patients, the T3 red cell uptake test was compatible with the final clinical diagnosis. In an experimental study of six patients, the T3 red cell uptake was the same immediately before and after performing an intravenous pyelogram. This lack of interference by organic or inorganic iodine was substantiated experimentally using a standardized system *in vitro* (Fig. 3).

SUMMARY

Experience in 176 patients indicated that the T3 red cell uptake test was useful in the diagnosis of thyrotoxicosis. The particular merit of the test was that it remained valid even after the administration of iodine-

containing medications or x-ray contrast media. Although reproducible and technically easy, the test was non-specific; several disease states other than thyrotoxicosis were associated with an increased uptake. Experimentally, the red cell uptake of T3 was demonstrated to be directly related to the serum thyroxine content.

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AN ELECTRON MICROSCOPIC STUDY OF THE VASCULAR SINUSES OF THE BONE MARROW OF THE RABBIT[1]

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In the bone marrow, as in the spleen, reticular cells line vascular sinuses and are the cells from which hematopoietic and other connective tissue elements may derive. Reticular cells are large mononuclear cells readily demonstrable by vital dyes. By light microscopy they are associated with reticular fibers and, in addition to their role in vascular sinuses, are considered to form a meshwork in which lie the remaining cells and extracellular ground materials of the marrow (1, 2, 3). In his electron microscopic study of the red bone marrow of guinea pigs, however, Pease (4) observed that extravascular reticular cells were rare if present at all.

The purpose of this paper is to describe the structure and relations of reticular cells in the marrow of rabbits as observed under the electron microscope, and to suggest that the vascular sinuses of this marrow are labile vessels capable of extensive modification in response to changing marrow needs. Observations upon fat cells, the other major connective tissue cell type about which the marrow is organized, are also presented. A small number of reticular cells were observed, in this study, as unassociated with sinuses. Littoral reticular cells of the vascular sinuses were the only elements comprising the vessel wall in most sinuses. Although they were typically associated with extracellular ground material, such sinuses lacked a basement membrane. In places, moreover, littoral reticular cells showed evidence of phagocytosis or cytopoiesis, highly modifying the structure of their vascular sinus, and obscuring its vascular character.

MATERIALS AND METHODS

Bone marrow was obtained from New Zealand albino rabbits weighing approximately 2 to 3 kilograms. During surgical anesthesia with intravenous sodium pentobarbital (5), marrow was removed from various portions of the humerus, femur, tibia and rib. Care was taken to avoid compressing the tissue. Usually, most of the circumference of the bony shaft of the femur or humerus was carefully removed in small pieces with rongeurs, exposing a cylinder of marrow. Within most of the shaft the

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internal surface of bone was smooth, so that the marrow could easily be lifted from the cavity. A cylinder of marrow, measuring about 0.5 cm. in diameter and up to 3 cm. in length, was freed with razor blade cuts and placed in 1 per cent osmium tetroxide buffered to pH 7.6 (6). After one hour the cortical 1 mm. of tissue was removed, cut into cubes, fixed an additional half hour, dehydrated in ethyl alcohol or methyl alcohol, and embedded in n-butyl methacrylate. Ribs were opened after a longitudinal split was started with a razor blade at the ventral end. The split was pried until the bone cracked along its entire length, yielding cephalic and caudal halves. As a result, a slender cylinder of red marrow, about 1 to 2 mm. in diameter, and about 1 to 2 cm. in length lay free in a half shell of bone at the dorsal end. Bone and marrow were dropped in fixative, in which the marrow usually floated free. Rib marrow was fixed for about 1½ hours, and while in alcohol was transected into smaller pieces suitable for embedding. Sections were cut for the light microscope and the electron microscope with the Porter-Blum microtome, the former sections stained with periodic acid-Schiff and hematoxylin (21, 22), the latter studied in an RCA-2E or, in a few cases RCA-3F, electron microscope.

OBSERVATIONS

In light microscope sections the walls of most sinuses consist of a single layer of reticular cells without a visible basement membrane (Plate I). This lining varied from a considerable thickness to a barely visible or even discontinuous membrane (Figs. 1 to 4, 15 and 18). The vessels were large in diameter but varied in width along their course. In general they did not follow a tortuous course but often their smooth contour would be broken as they bent about a cluster of erythroblasts, a fat cell, or other marrow elements (Fig. 9). Where a sinus ran next to a fat cell it was usually impossible to resolve as separate structures the cytoplasms of adjoining reticular and fat cells in the light microscope (Fig. 1). In a few places the lining cells of the sinuses appeared phagocytic. Occasionally vacuoles deeply stained in the periodic acid-Schiff reaction were present in the cytoplasm of the lining reticular cells (Figs. 2, 4 and 6).

Reticular cells apparently uninvolved in sinal walls were identified as large pale cells with large rather clear nuclei and abundant cytoplasm which was often extended in long slender processes. In areas crowded with other nucleated cells, a reticular cell could be identified only presumptively, its nucleus standing out as a clear vesicular structure, its cytoplasm clear and voluminous. A moderate number of reticular cells were phagocytic (Figs. 3, 19 and 21). In most cases they maintained their extended processes although the cells were no longer squamous.

Under the electron microscope littoral reticular cells presented a highly variable form (Plates II to IV). The cytoplasm was several micra thick or scarcely thicker than apposed plasma membranes. The plasma membrane was often thrown into irregular folds which displayed microprojections, or presented evidence suggesting pinocytosis. Occasionally large blebs of

ectoplasm were present at the cell surface (Fig. 22). The change in thickness of the cytoplasm could be abrupt. The luminal and abluminal surfaces of cytoplasm were little different in surface appearance except in relation to fat cells or other adjoining cells (vide infra). Evidence of phagocytosis was seldom observed in a sinal wall.

The texture of the cytoplasm varied. Frequently a finely granular, moderately dense material constituted the hyaloplasm (Figs. 9, 10, 14 and 21), while in other cases the cytoplasm between organelles was clear (Fig. 23). Mitochondria were present in moderate numbers. They varied in size, occasionally being large with complex cristae, but more often were small with few simple cristae. There were few mitochondrial granules. The endoplasmic reticulum was often abundant, consisting usually of great numbers of small smooth-walled vesicles (Figs. 10, 16 and 18). Only occasionally were granules, presumably ribonucleoprotein, associated with the endoplasmic reticulum. The Golgi complex, too, was well developed, represented as a system of small vesicles and closely lamellated smooth membranes (Fig. 20). Often several such groups were present in reticular cells, usually not far from the nucleus. Occasionally small, often irregular bodies of dense composition, presumably fat, were scattered in the cytoplasm of reticular cells (Fig. 10). In most cases this was bounded by a membrane similar to the endoplasmic reticulum. Infrequently what appeared to be fat was present in the nuclear envelope (Fig. 24), perhaps a vital demonstration of the continuity of the space in the endoplasmic reticulum with that of the nuclear envelope. Ferritin appeared to be scattered through the ground cytoplasm (and in some cases over the nucleus) but, as a rule, only in those calls evidently phagocytic (Figs. 19, 21). Such phagocytes were often rounded but persisted in maintaining long cytoplasmic processes.

The nuclei were of moderate size and regularly displayed a double membrane with "nuclear pores" (Fig. 20). Nucleoli were seldom observed. Often the nuclei possessed a deep cleft leaving a cytoplasmic *hof* which was usually free of membranous cytoplasmic elements. A small number of reticular cells did not appear associated with sinal walls. They were the same in form and appearance as parietal reticular cells.

Reticular cells were often applied to the circumference of fat cells, separated by extracellular connective tissue (Figs. 2, 20, 24 and 25) (vide infra). Such reticular cells were similar to those described above, but different in a tendency toward less surface activity, particularly at the surface lying toward the fat cell, and in a less fragmented, somewhat less abundant endoplasmic reticulum. In most cases such reticular cells clearly did form the lining of sinuses, interposed between lumen and fat cell. Such sinuses

typically exhibited fewer and smaller parietal apertures than others. Occasionally reticular cells lay back-to-back, separated by a ground substance in a manner reminiscent of the red pulp of the spleen (Fig. 17). Reticular cells also lay against megakaryocytes.

Fully developed unilocular fat cells were found in yellow and red marrow. Fortunately, in several thin sections the fat was almost completely dissolved away, leaving only a thin identifying rim of fat against the internal surface of cytoplasm and relieving the section of chatter (Figs. 20, 25, 28 and 29). The cytoplasm of fat cells contained moderate numbers of mitochondria, a few rather large with many cristae. The endoplasmic reticulum was rather scanty and without ribonucleoprotein particles. Fat cells showed no evidence of phagocytosis and appeared to contain no ferritin. In some cases the internal surface of cytoplasm of fat cells, facing on the globule of fat, was thrown into small folds and irregular processes (Fig. 29). However, the salient characteristic of the cytoplasm of fat cells was the presence of numerous small vesicles apparently involving the cell surface and suggesting pinocytosis (Figs. 26, 27).

Even in preparations where efforts were made to avoid compressing the tissue, moderate numbers of minute extracellular fat bodies were found in the sections (for example, Fig. 9).

At the junction of reticular cells there was frequently evidence of a condensed ground material (Fig. 10). Occasionally it had a fibrillar character but seldom were such fibrils clearly cross-banded. A ground material was often present between adjacent reticular cells and immature blood cells (Figs. 9, 10, 14, 15 and 22). In such ground material individual fibrils, as between reticular cells, were unusual, but between reticular cells and fat cells, fibrils were common. Indeed, the extracellular connective tissue between fat cells and any adjacent cell was rich in cross-banded and unbanded fibrils (Figs. 25, 26, 28, 29). The extracellular connective tissue so far discussed lay between adjacent cells. An exception was occasionally observed where a thin layer of ground substance of limited extent lay beneath a reticular cell with one surface uncovered by a cell (Fig. 22).

DISCUSSION

The findings in this study confirm earlier observations that the sinuses in the bone marrow consist of reticular cells (1, 7, 8) possess walls of variable thickness, and may be discontinuous (4). Most of them appear to lack a basement membrane. Sinuses in the marrow, moreover, appear similar to those in lymph nodes as described by Tanaka (9), Fresen and Wellensiek (10) and Donald and Coons (11). They also closely resemble the sinuses of the liver lobule in being formed only of littoral reticular cells and having apertures in the wall (12). The sinuses in the red pulp of the

spleen possess a fenestrated basement membrane but their lining, like that in the marrow, consists of reticular cells (13, 5). At least certain lymphatic capillaries are similar to the sinuses of the bone marrow in structure. Lymphatic capillaries in the diaphragm and the skin of the penis of the rat (14) and lacteals (15) consist entirely of endothelial cells. Their walls are variable in thickness, appear to lack a basement membrane and may be incomplete. Accordingly, the sinuses in hepatic lobules, red pulp of spleen, lymph nodes, and bone marrow, and lymphatic capillaries would be classified β-3-α in the system of Bennett, Luft and Hampton (12).

In the sinuses of marrow, sinal reticular cells are associated with a ground material lying between them and adjacent cells, similar to the material comprising basement membrane. The material may lie against the abluminal or, less usually, the luminal surface of reticular cells, or between them. Where sinuses lie against fat cells this ground material, variably rich in fibers, possesses the extent, constancy and histological character of a basement membrane. Lying beneath sinal reticular cells elsewhere it appears so limited and variable and so dependent upon the presence of other cells that, while of the same genre, it is difficult to recognize as possessing the histological status of basement membrane.

Splenic endothelium frequently displays a polarity absent in bone marrow, such as pinocytosis at the luminal surface, and a ground material below its basal portion. The polarity in splenic sinuses may be imposed by a basement membrane, the structure which, by its presence in the spleen and absence in the marrow, represents the most notable difference between the sinuses in these organs. A basement membrane would appear to be a relatively rigid structure. Thus the sinuses of marrow appear more pliant than those of spleen, conforming in contour even to such transient structures as erythrocytes. The view that the basement membrane controls the size of substances crossing the vessel wall has been presented by Pease (16) in relation to the glomerulus. In the spleen its wide fenestrations would minimize the filtration discrimination of the basement membrane—perhaps it represents an impediment merely to the passage of cells—but the presence of this membrane would provide the sinus stability and rigidity. The occurrence of a basement membrane in those sinuses lying in relation to fat cells may signify a more stable status for them—perhaps more nearly approaching simple conduits for blood—than for those lacking fat cells as a parenchyma. In the latter group, constituting most of the sinuses of red marrow, any discriminating capacity toward substances crossing the sinal wall perforce resides entirely in the littoral cell.

The basement membrane and other deposits of extracellular connective tissue represent the reticular "fibers" observed by light microscopy (17, 1, 2 and 3). In light microscopy their fibrous character is due to their linear

disposition. Under the electron microscope the material appears as a ground substance with variable numbers of collagenous fibrils present. Since this ground substance is always present and the fibrils may or may not be present the argyrophilia and other staining properties of reticular fibers in the marrow (and spleen) may be due to ground substance and not fibrils.

Blood was present within sinuses of the marrow and, for the most part, erythroblasts, myelocytes, and other hematopoietic and connective tissue elements were outside. But blood was also present outside such vessels and occasionally myelocytes and, fairly frequently, erythroblasts, were inside.

The bone marrow, the red pulp of the spleen, and the lobules of the liver, served by reticuloendothelial sinuses, lack lymphatic capillaries. Perhaps these sinuses, as suggested earlier (5), carry lymph as well as blood and in this sense constitute lymphatic vessels as well as blood vessels. Their structural similarity to lymphatic capillaries has been noted above.

Sinuses are inherently unstable vessels because reticular cells which form their wall are motile cells, having secondary functions incompatible with their function as endothelium. For example, when phagocytosis is forced upon the reticuloendothelial system the endothelium of sinuses may leave the wall with gaps forming as a result. Again, lining reticular cells may differentiate into other cell types and leave the sinal wall. In the case of the liver the outlines of the sinuses, and possible blood flow, may be maintained by the disposition of parenchyma as cords. In the spleen the outline of a vessel whose endothelium has become reactive is maintained, at least for a time, by its basement membrane, although the vessel may be so changed in appearance that it no longer looks like a blood vessel. Such transformed vessels are considered the Billroth cords of conventional histology (5, 13). But in the bone marrow, except as they course among fat cells, sinuses lack even the fenestrated basement membrane of splenic sinuses and bear no consistent histological relationship to such elements as hepatic epithelium or splenic cords. In the bone marrow, if sufficient numbers of sinal reticular cells give up their endothelial functions and are not replaced, their sinus may become grossly incompetent or even disappear, leaving behind as the vessel's only trace, scattered phagocytic cells, cytopoietic loci, or isolated endothelial elements. This process may account for the presence of reticular cells unassociated with sinuses. So simple may be the wall of a sinus, consisting only of a single layer of flattened, discontinuous reticular cells, that the distinction between an alignment of a few extravascular reticular connective tissue cells and a vessel with major portions of its endothelium out of line, may not exist. In many places, moreover, because of the incompleteness of the wall and the lack of luminal-abluminal polarity in the endothelium it is impossible to distinguish intravascular from extravascular. It appears to the author that the vascu-

lar sinuses of marrow represent a system of fugitive vessels, capable, through the motility and multipotentiality of their reticular cell wall, of changing course, or varying the completeness of their wall—even of forming and disappearing—in response to changing requirements for such marrow functions as erythropoiesis, myelopoiesis, antibody formation, phagocytosis, lymphatic drainage, blood flow, and delivery of blood cells to the circulation, or sequestration of cells from the circulation. In the process, part of a sinal wall may become detached and isolated, and reticular cells near the course of a forming sinus but currently uninvolved in a sinus, become incorporated as lining cells. Any of the space of the marrow may rapidly become part of the vascular system and the concepts of intravascular and extravascular, worn in the context of conventional vessels, is inapplicable to this labile complex of cells.

SUMMARY

Reticular cells in bone marrow form vascular sinuses. They may become motile cells and may have such additional functions as phagocytosis and cytopoiesis. These functions may be incompatible with their endothelial role. In proportion to the number of cells which assume such secondary functions and are not replaced, segments of a sinal wall may disappear and the vessel be rendered incompetent. Those isolated endothelial or phagocytic reticular cells observed in marrow may represent the only remnants of transformed vessels.

The sinuses of the marrow, except those in relation to fat, had no basement membrane. This represented the most significant difference between these vessels and the sinuses of the spleen.

A moderately dense layer of ground substance, similar to basement membrane, lay subjacent to reticular cells over a limited area, one surface covered by the reticular cell, the other by an immature blood cell or connective tissue cell, or unusually, free. This ground material, while of the same character as basement membrane, lacked sufficient extent, uniformity and constancy to designate as a basement membrane. The ground substance near fat cells was relatively rich in fibrils.

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PLATES I TO X

PLATE I

Figures 1 to 7 are photographs of bone marrow from the proximal femur or proximal humerus, embedded in methacrylate after fixation in osmium tetroxide, sectioned at 2–3μ, stained by the periodic acid-Schiff method, and photographed at a magnification of $\times$ 1300.

FIG. 1. A sinus curves from the lower border of the photograph on the left side up to the right corner where it lies between two fat cells. The endothelium is irregular, varies in thickness, and appears incomplete. It appears to lack a basement membrane. Where they touch it is not possible to resolve the cytoplasm of the fat cell from the endothelium of the sinus (upper arrow). The lower arrow indicates a portion of endothelium. That part of the endothelium lying over the first three letters in "sinus" is incomplete.

FIG. 2. A clearly defined sinus is labeled. Note the variation in thickness of its endothelium. It appears to be incomplete (for example beneath the letters "i" and "n") and to lack a basement membrane. Portions of two fat cells are present and meet along their perimeter. They contain one large locule, and, in the peripheral cytoplasm along their upper perimeter in the photograph, several smaller locules. The fat has been dissolved away as in Figure 1. A reticular cell lies on the circumference of the fat cell on the right (arrow). Part of its cytoplasm appears loosely applied upon the surface of the fat cell. Compare this cell with the reticular cell in Figure 20. In the endothelium of the labeled sinus, and in the cytoplasm of this reticular cell at an indentation in the nucleus, inclusions stained with the periodic acid-Schiff reaction are present. See Figures 3, 4, 6, 14 and 19 in this connection. Note the strand of reticular cell cytoplasm at the upper arrow, marked "r".

FIG. 3. Several fat cells are present here, their fat incompletely dissolved away. Most of them contain a large locule of fat and several very much smaller ones. Note that the contour of several of the fat cells is somewhat irregular. A large sinus is present in the left lower quadrant of the photograph. Two of its endothelial cells are indicated by arrows beneath the word "sinus". The extended cytoplasm of the upper one shows well. The wall of this sinus consists only of endothelial cells. It appears incomplete. Sinuses, defined to varying degree, appear elsewhere. There appears to be one between two fat cells. Part of its wall is indicated by the top-most unlabeled arrows. A relatively poorly delineated vessel appears to lie in the lower right corner, part of its endothelium (containing PAS-stained inclusions) indicated by the arrow. Note the phagocytic cell at the right upper margin of the photograph, its nucleus labeled "r".

FIG. 4. A slender sinus runs from the top of the photograph to the bottom, dipping out of the section along its course. Vertical arrows lie in top and bottom segments of the vessel, the lower arrow pointing at an endothelial nucleus which lies just at the level of the vessel's narrowing and the disappearance of the lumen. Note the PAS-stained inclusions in the sinal endothelium. Several are in the region indicated by the arrow marked "p". Others are above the endothelial nucleus (n) and above the endothelial nucleus at the vessel's narrowing. A small angular cell (its nucleus labeled "fc") containing fat in many locules, is present in the lower half of the photograph to the right of the sinus. It has a long slender cytoplasmic process which extends almost directly toward the top of the photograph, and another which runs to the bottom (unlabeled horizontal arrows).

In *Figure 6* another well defined sinus is present. Its wall too consists of the thickness of a single endothelial cell which varies in thickness and, in places (unlabeled arrows) is absent. The endothelium contains several PAS-positive bodies, to both sides of the leftmost arrow and just to the right of an endothelial nucleus at the arrow marked "p".

FIGS. 5 and 7 contain fat cells which are irregular in shape and contain locules of varying size. Most of the locules appear spherical but a few have an irregular contour. In several of the larger locules some evidence of fat persists as central densities. In Figure 5 the nucleus of the fat cells is labeled "fc". The larger cell is very large, occupying the lower ⅔ of the photograph, with locules above and below the nucleus. A phagocytic cell, its nucleus labeled "r" lies in the upper third of the field.

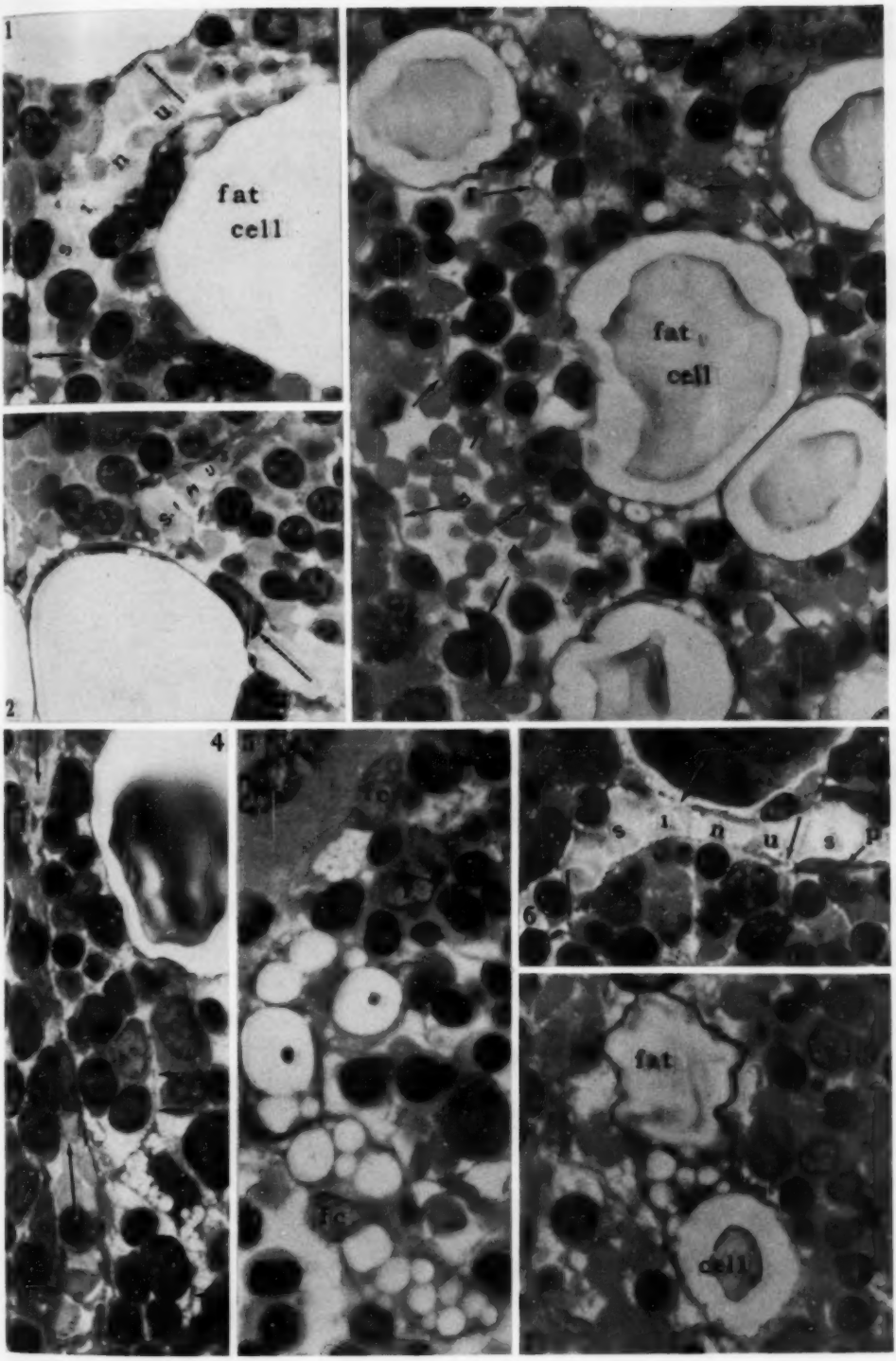

1
fat
cell
2
fat
cell
4
fc
fc
6
fat
cell

PLATE II

FIG. 8. A small sinus in red costal marrow. Endothelial junctions are indicated by luminal arrows. Note the variations in thickness of endothelium. There appears to be no basement membrane, but note the ground substance (gs) between the endothelium and an adjacent myelocyte. Note the vesicles in the endothelium and the large vacuole (v) containing granular material. Compare the latter to that in Figures 14 and 19. There are several reticulocytes, displaying mitochondria, outside the vessel and one inside. Approximately × 4000.

FIG. 8. Electron micrograph of a thin section of the glomerulus of a rat kidney. Fixation in osmium tetroxide. Magnification 10,000. The glomerular capillary wall consists of a thin layer of cytoplasm (c) and a basement membrane (bm). The latter is separated from the capillary lumen by the endothelial cell (e). The epithelial cell (ep) is separated from the basement membrane by a space (s). Fenestrae (f) are present in the endothelial cell. Glomerular space (gs).

PLATE III

This plate consists of segments of the walls of sinuses.

FIG. 9. Immature red cells are in the lower portion of the photograph. The sinal wall consists of reticular cell cytoplasm. Note that, especially in its middle portion, each surface of the endothelium is irregular. In the portion of the wall at the left hand margin of the photograph it appears that the cytoplasm of two reticular cells are apposed and form the wall. Note how the wall curves about the red cell at the right border of the figure. Subjacent to the endothelium at the right of the photograph, between it and the red cell, lies a ground substance (arrow). × 25,000.

FIG. 10. This is a segment of a sinus with relatively few apertures in its wall. Portions of two erythrocytes (E) lie along the lower margin on the right. A junction of endothelial cells may be seen in the photograph (upper 3 arrows). Here the cells are separated by a ground material of moderate density, varying in concentration and, at the lower arrow, extending to the lumen. A similar ground substance is present to the right along the luminal-abluminal surfaces of the endothelium. There are no fibers visible in this ground material. The upper surface of the vessel is more irregular than the lower one, and numerous small vesicles and smooth-surfaced endoplasmic reticulum are present in the cytoplasm. Two mitochondria (M) are labeled. Note the variations in thickness of the vessel wall. × 23,000.

FIG. 11. This segment of a sinal wall is less rich in membranous components than that in Figure 10. It is slender and its ground cytoplasm is finely granular and moderately dense in places and rather clear elsewhere. Note the mitochondrion (M). A portion of an erythrocyte (E) is at the lower margin in the sinal lumen. × 35,000.

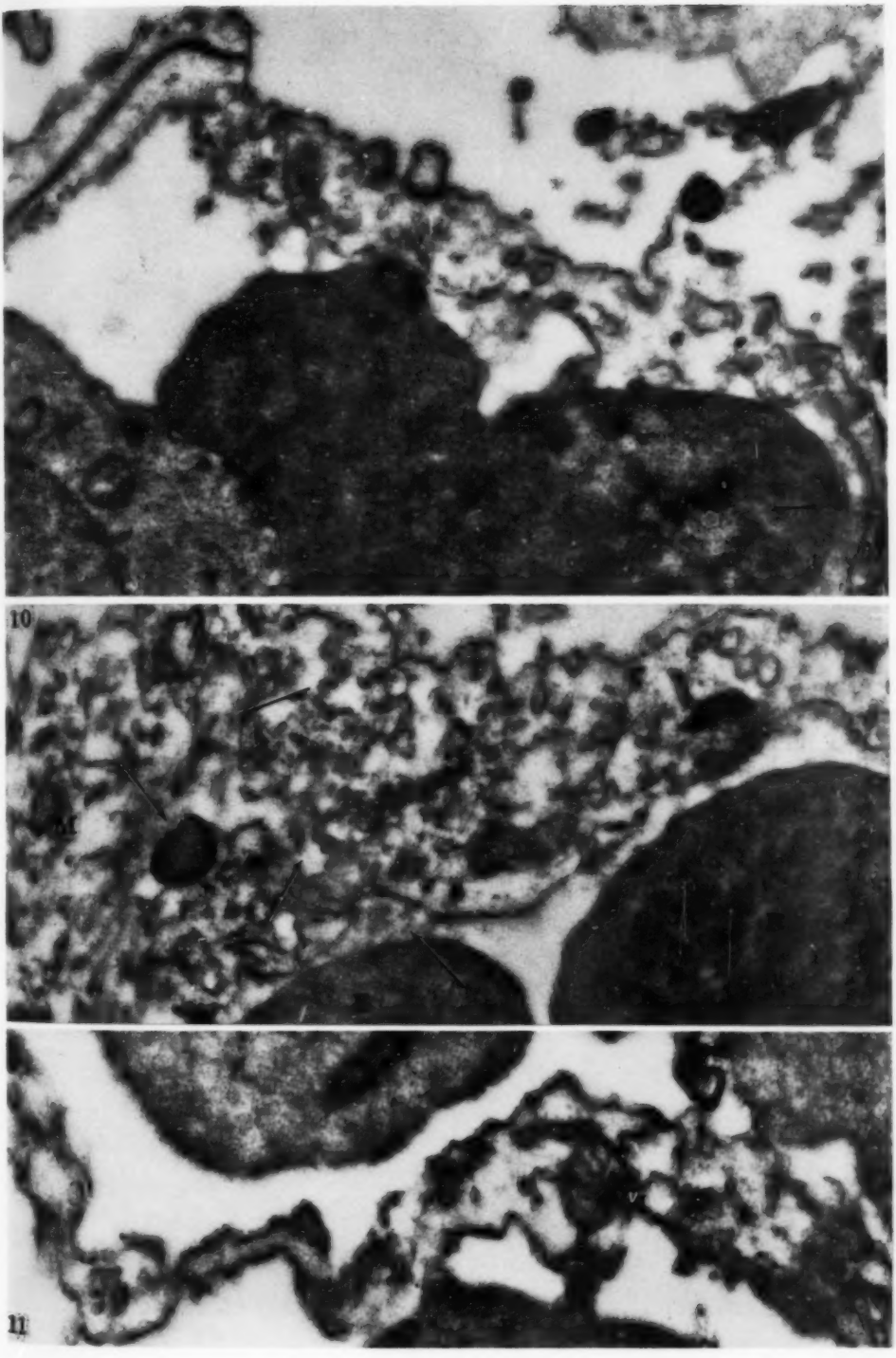


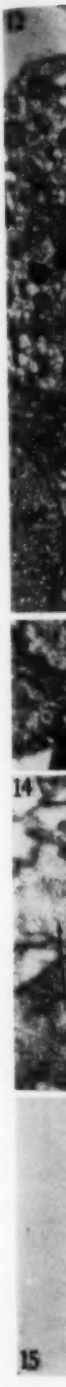
PLATE IV

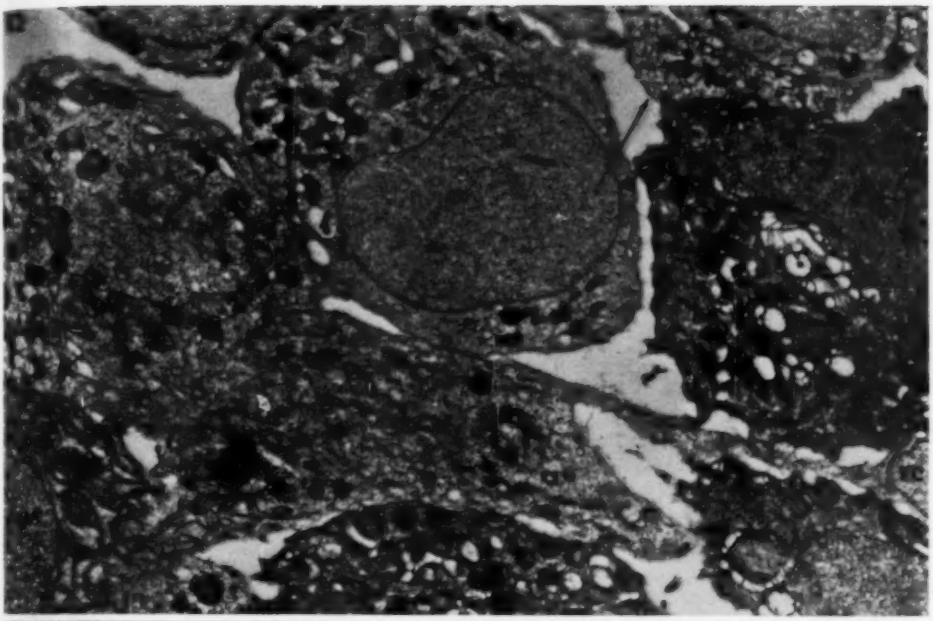
FIG. 12. Portions of several myelocytes are present in this field. A group of plasma cells was present on the left, a portion of one of which (labeled "pc") is included in the photograph. A reticular cell, in endothelial form is on the right of the field, its nucleus marked "rc". It touches a large cell which may be a reticular cell undergoing transformation. Near the top of the field, at the arrow, is a small portion of cytoplasm. × 6000.

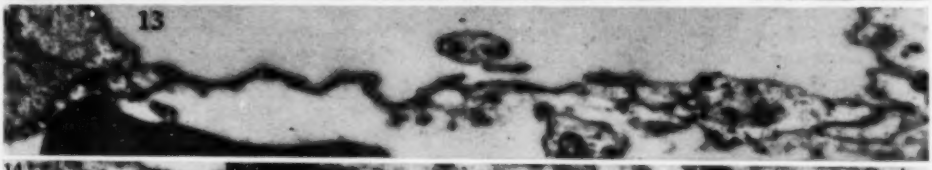
FIG. 13. A portion of a sinal wall demonstrating, in the left half of the photograph, how thin an intact endothelium may be. This portion of the vessel wall consists of little more than apposed plasma membranes. A portion of an erythrocyte lies beneath it.

FIG. 14. A portion of a sinal wall. The single thickness of endothelial cell comprising the wall presents irregular surfaces. At the right end of the photograph, between the arrows, it is quite attenuated. Note the vacuoles containing a granular material on the left at the arrows. Possibly such structures account for some of the PAS reaction as seen in Plate I. Compare with Figures 8 and 19. Note also the large endothelial process (P) projecting into the lumen. It contains some finely fibrillar material and lies against an immature erythrocyte, separated by a thin layer of ground substance. The vessels of which this segment is a part have a markedly incomplete wall.

FIG. 15. A segment of an incomplete sinal wall. Note the aperture between reticular cells, and a ground material (arrows) between the reticular cells and the underlying immature blood cell. × 22,000.



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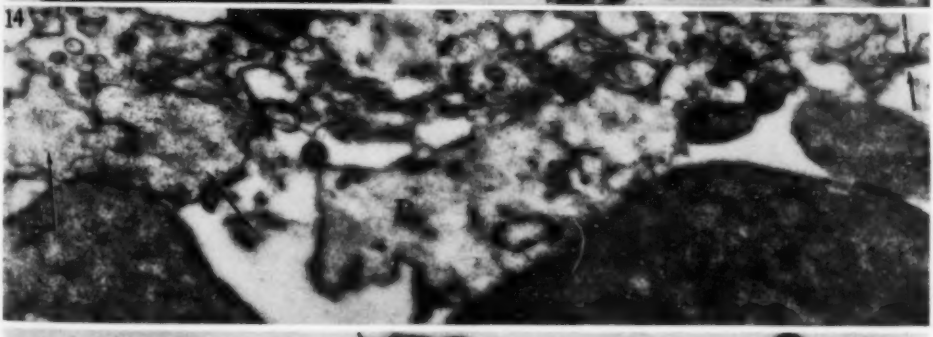
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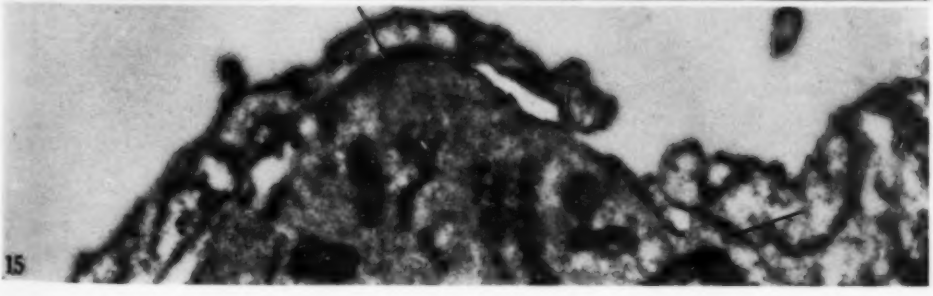

15

PLATE V

FIG. 16. A portion of an endothelial reticular cell showing a great number of vesicles apparently associated with pinocytosis. × 42,000.

FIG. 17. Mature red blood cells are present above and below the horizontal segment of sinal wall. It appears that two endothelial surfaces, each complex, is separated by a ground substance, marked in three places "gs". × 29,000.

FIG. 18. A small segment of a sinal wall showing great complexity. A portion of a reticular cell (rc) is on the left, another (rc) on the right, the two separated by a gap. Below the right portion of a reticular cell are several additional segments of reticular cell cytoplasm. Note the small isolated segments (arrows) forming a luminal surface in the right lower quadrant. Another lumen is along the upper border of the photograph above the labeled reticular cells. × 42,000.

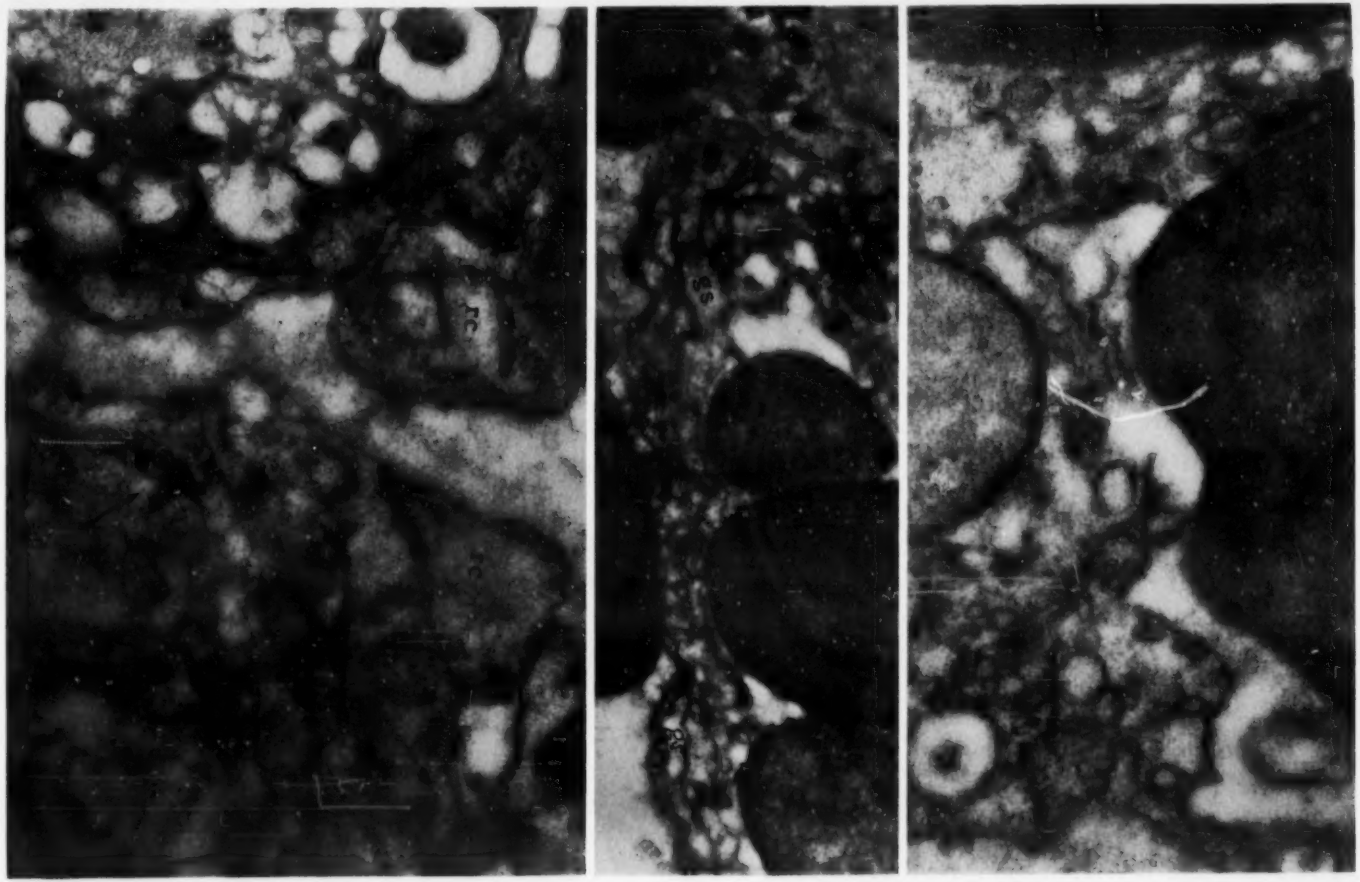


PLATE VI

FIG. 19. This is a phagocytic reticular endothelial cell which has retained its position in the wall of a sinus. Its nucleus is at the bottom of the plate (EN). The lumen of the vessel is marked "SL". The junction of this cell with its neighboring reticular cell is marked by arrows and contains ground substance. This cell, as in Figure 23, contains ferritin. Note the large central vacuole just above the nucleus filled with a moderately dense ground material. By comparing sections in the light microscope with the electron micrographs, it appears that such vacuoles may constitute some of the PAS-stained vacuoles in the light microscope sections. See Figures 3, 4, 6, 8 and 14. × 36,000.

SL
SL

PLATE VII

FIG. 20. This figure illustrates a characteristic relationship between reticular cells and fat cells. A fat cell lies in the upper portion of the field. Most of its fat has been dissolved away but a rim (arrow "F") persists closely applied to the internal surface of the cytoplasm (arrow "C"). The cytoplasm has been reduced to a narrow rim. The reticular cell lies upon the circumference of the fat cell and a variably concentrated ground material (arrow "GS"), constituting a basement membrane, separates the cells. The cytoplasm of the reticular cell is rich in endoplasmic reticulum and contains several inclusions. The nucleus of the reticular cell is labeled "RN". Its double membrane shows clearly and several "nuclear pores" may be seen (arrows). This reticular cell contributes to the wall of a sinus, whose lumen is marked "SL".

Compare this electron micrograph with light micrographs Figures 1 and 2 and with electron micrograph Figure 24. × 30,000.

FIG. 21. A phagocytic reticular cell. This photograph is in the juxtanuclear region. The cell extends long cytoplasmic processes from the top of the photograph in the direction of the arrows. Note the diversity of intracytoplasmic inclusions. Ferritin appears present throughout the cell including the nucleus but excluding mitochondria and some clear vacuoles (see also Fig. 19). Several of the mitochondria are labeled "M". In phagocytic cells they are typically far more pleomorphic than in other reticular cells. × 36,000.

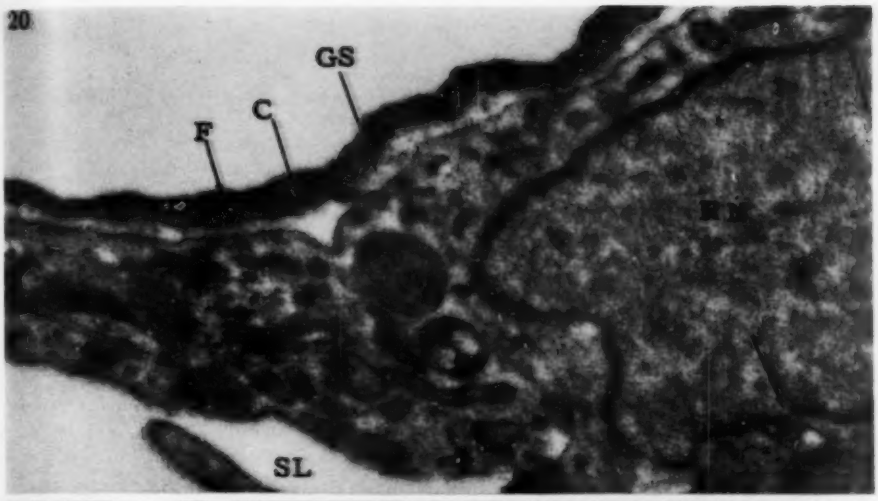

GS
C
F
SL

PLATE VIII

FIG. 22. Most of the field is occupied by a sinus part of whose endothelium has become phagocytic (marked "PE"). The sinal lumen is lettered and extends in the direction of the arrows. Note that on the left, the transformed endothelial cells encroach upon and almost obliterate the lumen. Note also, the vesicular, bleb-like cytoplasmic processes of the endothelium (labeled "B"). Between the endothelium and a subjacent myelocyte is a thin layer of ground substance (upper "GS"). Below this area is a portion of subendothelial ground substance whose reverse surface is free of a covering cell (lower "GS"). On the other side of the vessel is a cell junction ("J") running more or less tangential to the lumen. Compare this with Figures 8, 9 and 14. × 12,000.

22
GS
GS
N
L
B
B
B
L U
B
P

PLATE IX

The fields in Figures 23 and 24 were taken from proximal femoral marrow.

FIG. 23. An erythrocyte lies in what appears to be a slender portion of a sinus, occupying virtually all of the lumen. Note the reticular cell cytoplasm in the lower right corner of the photograph (arrow) and compare with the left and right thirds of the endothelium of Figure 19, and with the similar junction in Figure 22.

FIG. 24. This field consists of portions of two reticular cells and a fat cell. Only a small part of the fat cell is present, in the lower right corner, where a small segment of peripheral cytoplasm (fc) and, in the very corner, the dark fat, may be seen. Portions of one reticular cell on the right and another one on the left, are separated from the fat cell by ground substance (gs) constituting a basement membrane. The reticular cells themselves have an irregular junction (lower 3 arrows) of cell membranes without interposed ground substance. A dense material which appears to be fat, lies within the nuclear envelope at the upper arrows. These reticular cells are in the wall of a sinus, whose lumen is marked "sl". See text. Compare with Figure 20. × 23,000.

FIG. 25. This field consists of portions of two fat cells which touch along part of their circumference. The fat has been dissolved away (F) and a fibrillar material lies between the fat cells (arrow on the right). In the original photograph cross striations may just be recognized in some of these fibrils. Note the cell, which appears to be a reticular cell, lying between the fat cells and similarly attached to the fat cells by fibrils (arrow on the left).

This field is taken from distal femoral marrow which was grossly pink, tinged with fat. Compare with Figures 2, 3, 26 to 29. × 32,000.

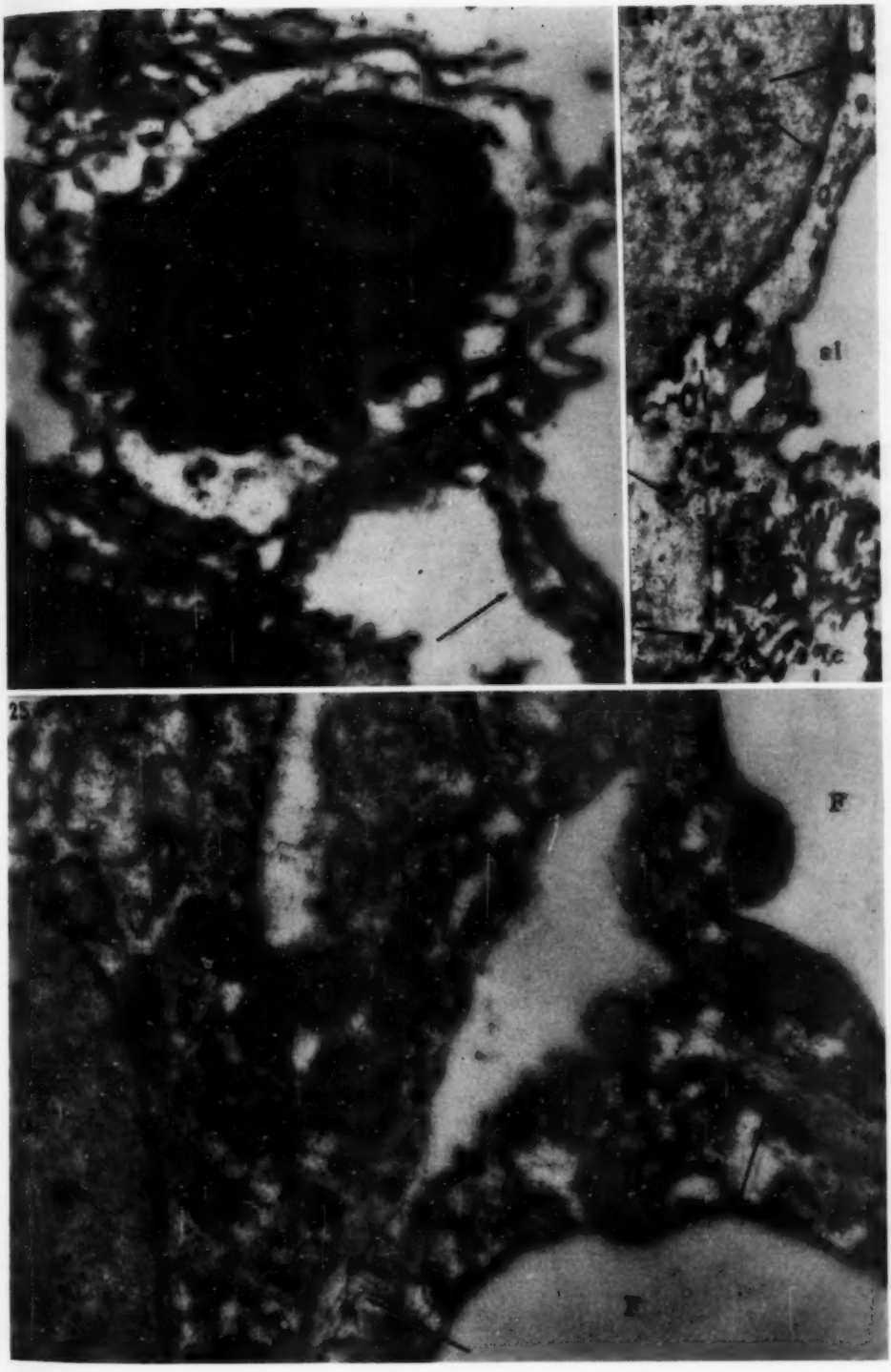

el
F

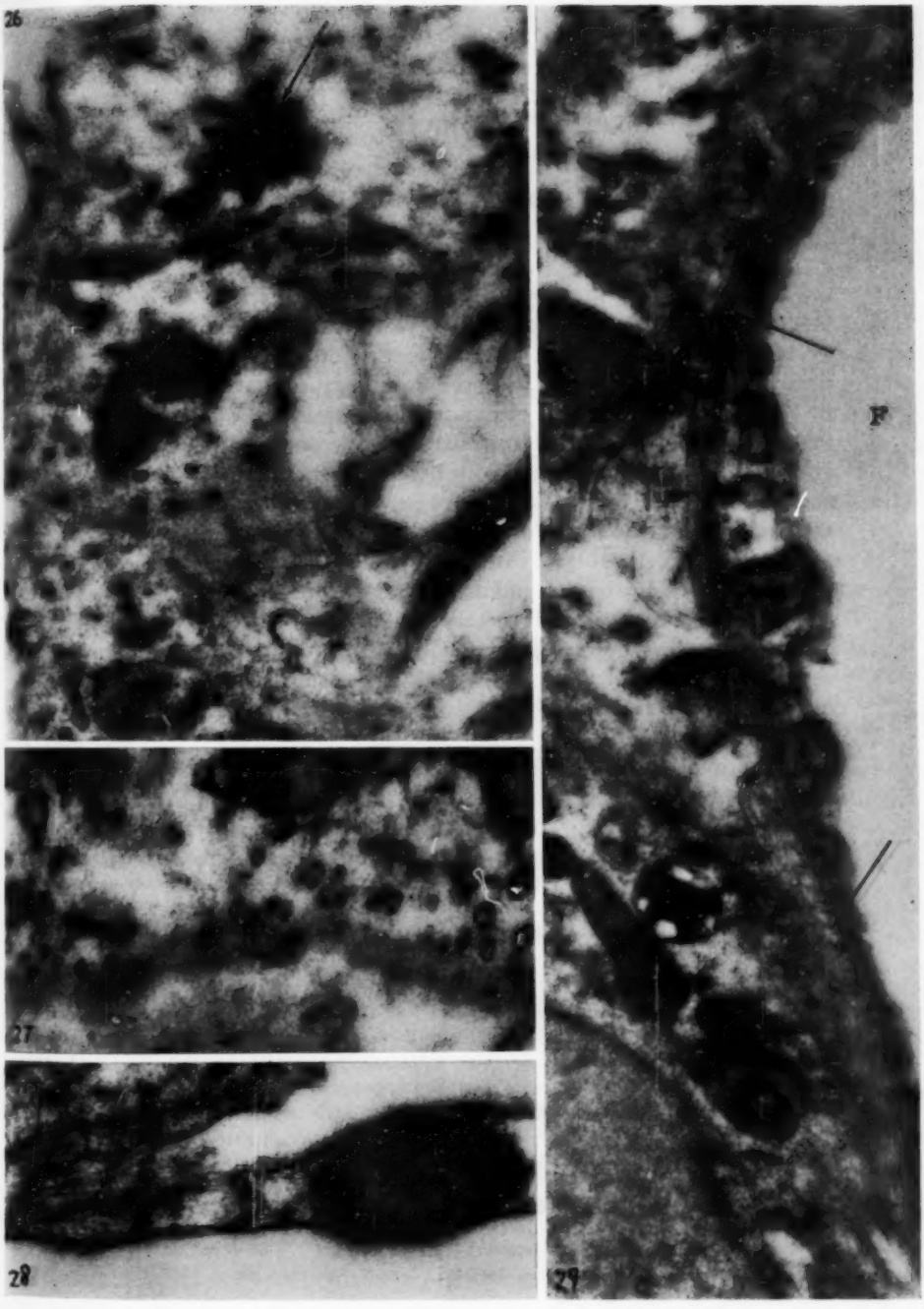
PLATE X

FIG. 26. This field is taken from the yellow marrow of the distal part of the tibia. A portion of the marrow rim of a fat cell underlain by fibrils (to the right) occupies the field. It contains a droplet of fat in its cytoplasm (arrow) in addition to many small vesicles, apparently associated with pinocytosis. See following figure and compare with Figure 16. × 32,000.

FIG. 27. The surface of a fat cell showing many small vesicles in their relation to the plasma membrane. × 37,000.

FIG. 28. Here a portion of a unilocular fat cell, near the top of the photograph, lies upon an hematopoietic cell. Except for a very thin layer against the internal surface of cytoplasm most of the fat in its locule (F) has been dissolved away. The fat cell contains a large mitochondrion. Note the fibrillar extracellular connective tissue separating these cells (arrow). No crossbandings are visible. × 37,000.

FIG. 29. A myelocyte ("M") lies next to a fat cell in yellow bone marrow. Again the fat has been dissolved from the section (F). The internal surface of the fat cell cytoplasm is irregular. Extracellular ground material and fibrils are interposed between the cell types (lower arrow). Most of the fibrils in this field are simple; some, however, are cross-banded with a major periodicity of 640 Å (upper arrow). × 36,000.


26
27
28
29
F

ALBUMIN, CONNECTIVE TISSUE, AND THE BLOOD-BRAIN BARRIER[1]

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INTRODUCTION

The concept that a barrier exists between the blood and the brain has been accepted for 75 years, but the nature and location of that barrier have eluded definition as more and more substances have been used to test it. Ehrlich is credited with originating the concept in a study published in 1885 in experiments with injected dyestuffs, and he and others in later years were able to make the now well-known generalization that acid dyes are as a group barred from the brain (1 to 3). A burgeoning interest soon led to investigations of many substances other than dyes, and by 1942 it was possible in a review of the literature to cite experiments using toxins, venoms, viruses, antibodies, and drugs; and to note some controversy about the relative roles played by the charge, size, lipoid solubility, and degree of serum protein binding of these molecules (4). More recent studies have used radioactive ions and metals, such as palladium and silver, with an increasing emphasis on demonstrating a precise histological site of the barrier in or around the cerebral capillaries (5 to 9). Although one may well feel timorous about adding yet another molecular type to an already long list of substances, serum albumin appears interesting both in its behavioral similarities to some of the compounds used by other investigators, and also on its own account, as a physiological molecule in the colloid size range.

MATERIALS AND METHODS

Human serum albumin tagged with I^{131} was purchased from the Squibb Company (trademarked ALBUMOTOPE). On dialysis it yielded a maximum of 1.5 per cent of its activity as the unbound iodide, and was therefore used as purchased. The albumin was injected via the heart into the blood stream of each of six adult cynomolgus monkeys (Macaca, irus group, Philippines) and after a specified interval of time the anaesthetized animals were perfused with formol-saline; the appropriate tissues were then removed, immediately weighed, and counted inside plastic vials in a well-type scintillation counter. Cerebrospinal fluid and bloods, when drawn, were pipetted in duplicate and similarly counted.

One additional monkey, also given iodoalbumin, was perfused with normal saline (no added fixative) after which its entire cerebral cortex was removed and prepared

[1] Aided by a grant from the National Foundation, Inc.

[2] Henry Strong Denison Scholar, 1960–61.

for electrophoretic analysis. Brief homogenation in a Waring blender was followed by 20 minutes of sonication, after which the homogenate was ultracentrifuged at 30,000 r.p.m. for one hour and the clear supernate removed. One hundredth ml. aliquots of the supernate as well as the original iodoalbumin were subjected to electrophoresis on separate paper strips for 14 hours in a Spinco apparatus (5 amps; veronal buffer, pH 8.5, μ 0.06) and the strips were then analyzed for the distribution of protein and radioactivity.

Estimation of the proportion of collagen in several of the tissues that were counted was performed microscopically on 7 μ Mallory-stained sections, which were randomly scanned through an eyepiece with four pointers, and the 'hits' on collagen tallied as a per cent of the 'hits' on the whole tissue. Four monkeys (not those used in the above experiments) were studied in this way. The method is that of Chalkley, who has discussed its rationale, technical details, and general limitations (10); as applied here the chief limitation is that the density of the collagen in various tissue sites is not precisely reflected by its staining characteristics.

RESULTS

The results of the albumin experiments are shown in Table I. All values represent counts per minute per milligram of tissue (or fluid) and are cor-

TABLE I

Tissue Counts Compiled from Six Monkeys Following Administration of I^{131} Albumin

	Monkey Number						Mean Value	
	58-4	58-6	59-1	59-3	59-4	59-10	6 hr.	Total
Dose (microcuries)	750	670	900	970	850	980		
Weight (lbs.)	4.5	4.0	3.0	2.8	2.8	4.6		
Exper. time (hrs.)	24	24	24	6	6	6		
Tissues								
CSF				8.60	2.47	10.6	7.23	
Caudate nucleus		7.48	3.60	3.77	3.53	4.46	3.92	4.57
Corpus callosum		2.93	5.13	3.80	3.71	2.37	3.76	4.05
Cerebral cortex								
pial surface		8.05	8.90	6.26		5.47	5.89	7.17
deep tissue	2.15	3.78	5.03	5.68		3.60	4.64	4.05
Cervical cord (stripped)	1.94	2.54	2.24	7.53	7.20	7.47	7.40	4.82
Cervical pia-arachnoid			23.4	29.9	46.1	53.8	43.3	38.3
Medulla oblongata								
pial surface		9.55	6.90	7.10	4.70	8.77	6.86	7.40
ependymal surface	4.96	3.48	7.16	5.60	2.32	6.21	4.71	4.96
deep tissue		1.77	4.39	3.41	3.24	9.68	5.44	4.50
Area postrema		51.2	29.3	41.3	108		74.7	45.9
Pineal body	100	27.3	24.8	40.5	47.6		44.0	48.0
Neurohypophysis		88.8	80.0	151	55.4	109	105	96.8
Liver			20.8	44.6		59.0	51.8	41.4
Sciatic nerve		97.8	50.0	49.1	64.1	72.8	62.0	66.8

All counts have been made comparable by a dose per weight calculation, and are expressed in cpm/mg. tissue.

rected to the time of injection. Counts for the different animals have been made comparable by a dose per weight calculation, and the individual doses and weights are also included for each animal. The figures in the row marked 'EXPER. TIME (hrs.)' indicate the interval between the time of injection and the time of sacrifice, i.e. the length of the experiment. Although the last three experiments were curtailed to six hours in order to minimize the variable introduced by metabolism of the injected albumin, the mean values can be seen substantially to agree with those calculated from the group as a whole.

Several assumptions about the injected iodoalbumin will be discussed before any attempt is made to interpret these experiments. The first of these is that human albumin, which has further been radioiodinated, is handled like homologous albumin by the monkey into which it is injected. Investigations designed to test the general case among several species-pairs, including human I^{131} albumin injected into rabbits, have demonstrated identical or nearly identical blood disappearance curves over a period of many days (11). In view of the similar disappearance curve obtained under the conditions of the present experiments using a closely related human-monkey species pair, and because the liver seems not to have been phagocytically active in these monkeys (compare with cervical meninges in Table I), the validity of the original assumption has been accepted.

A second assumption is that perfusion of the animal is equal to the task of adequately ridding the vessels of their blood, i.e. that the tissue counts do not merely represent residual intravascular albumin. Microscopic sections failed to reveal more than a very rare capillary containing stained erythrocytes or proteinaceous material. Furthermore, the perfusion was in one animal immediately preceded by an extensive dextran transfusion that reduced the blood activity by a factor of seven, without any significant lowering of tissue counts.

A third assumption is that the radioactivity counted in the tissues is still bound to the albumin, and not free, or even bound to some other compound. The electrophoresis experiment using cerebral cortex was included as a check on this point, although it must be understood that a rigorous proof of the hypothesis would require similar analyses of each tissue counted. This has not been done because the small size of many of these tissues presents a technical impasse to extraction and electrophoresis. Results with the cortical extract, however, revealed that 90 per cent of its activity migrated at the same speed as the albumin peak of the simultaneously tested original iodoalbumin. The remaining 10 per cent was spread out more or less evenly among the slower moving globulins. Whatever the explanation for this lagging activity—and up to 5 per cent of it might be accounted for by radioactive impurities in, and metabolism of, the

injected material (11)—the point to be emphasized is that 90 per cent remains bound to albumin, and therefore permits the counts of this tissue to be regarded as representative of the albumin content.

With reference to the data in Table I, it should be stated at the outset that the concentration of albumin in the central nervous system as a whole is considerably less than in other tissues. This roughly agrees with a previous report on albumin distribution based on somewhat different experimental conditions in the human (12). Among the several wholly neural tissues that were studied, one finds a rather uniform distribution of albumin activity; there is no difference between white and grey matter (thus the counts from the nearly pure grey caudate nucleus, the purely white corpus callosum, and the mixed cortical and cord tissues are all about the same). Also, though not included in the table, there is only negligible variation in counts of different samples of the same tissue; for example, among five different areas of cerebral cortex. Nor does there appear to be any appreciable difference between the activity of surface tissue, close to the cerebrospinal fluid, and underlying deeper tissue—shown in Table I for cortex and medulla. And finally, the cerebrospinal fluid itself contains about the same albumin concentration as all these various examples of neural tissue, which, in view of the much higher levels found in the circulating blood, suggests that the blood-brain and blood-CSF 'barriers'—which have come to be distinguished as separate systems—are here behaving similarly. (It says nothing about the third system between CSF and brain, but a recent experiment in which iodoalbumin was injected into the cisterns of cats and followed by autoradiographs, showed more activity in subependymal, subpial and meningeal sites than in the deeper neural tissues (13). This appears to have been largely if not entirely the effect of diffusion and indicates the absence of any surface barrier to albumin; it is therefore at least consistent with the observations presented here.)

Besides the CSF and those purely neural tissues that were sampled, however, it was decided to examine a few tissues which although within the CNS cannot be said to be typically neural, and which belong among a group of tissues that have for years occupied the attention of investigators in this field, particularly those who have worked with dyes. These are the tissues which have been called exceptions to the concept of a blood-brain barrier insofar as they are stained with acid dyes that leave the rest of the CNS uncolored: The neurohypophysis, saccular eminence, supraoptic crest, intercolumnar tubercle, area postrema and pineal body (14). More recently they have been observed to behave similarly toward silver, which is detectable both with the light and electron microscopes (7 to 9). The more easily accessible of these tissues were included in the present experiment (neurohypophysis, area postrema, and pineal body) and may be seen to

contain a considerably higher albumin concentration than neural tissue, just as they have been noted to contain more dye and more silver. Also included for comparison with this group is the sciatic nerve, an example of peripheral nervous tissue and therefore not affected by any blood-brain barrier mechanisms as presently understood.

DISCUSSION

One characteristic shared by all of the so-called exceptional tissues is their remarkable quantity of connective tissue compared with the rest of the CNS. In an effort to quantitate this observation, Mallory-stained sections of these tissues, together with a few representative neural tissues, were examined according to the Chalkley method outlined earlier, with the results shown in Table II. It is important to remember that collagen is only a rough measure of connective tissue space; yet despite this and the previously mentioned technical limitation of evaluating the collagen itself, there is a notable correspondence between the proportion of collagen and content of albumin in the CNS tissues analyzed.

It may still be asked whether such a correspondence implies a real relationship, or mere coincidence. In measuring central nervous system connective tissue one is really measuring vascular connective tissue, for it is virtually non-existent apart from the vessels. In neural tissue the vascular endothelium abuts neuroglia, with no intervening connective tissue, whereas in the special areas—pineal, etc.—the vessels are heavily invested with a connective tissue sheath. It would therefore be reasonable to think that the net movement of the iodoalbumin is outward through the capillary endothelium until an equilibrium is reached, that this equilibrium is mainly determined by the volume of the extravascular space capable of containing the albumin, and that the loose perivascular connective tissue

TABLE II

Collagen Proportion Compared to Albumin Activity in Different Tissue Groups

	% Collagen	Albumin Activity
Typical CNS tissue (no perivascular collagenous tissue)		
Cervical cord	2.3	4.82
Cerebral cortex	3.4	4.05
Caudate nucleus	3.5	4.57
Atypical CNS tissue (perivascular collagenous tissue present)		
Area postrema	10.6	45.0
Pineal body	11.5	48.0
Neurohypophysis	14.0	96.0
Peripheral nervous tissue (Dense collagenous tissue)		
Sciatic nerve	28.4	66.8

qualifies as such a space, whereas the tightly packed neural tissue does not. Equilibrium will therefore be reached in the 'special' tissues after a relatively large albumin crossage, and in the rest of the CNS after a relatively small crossage.

Stated in this form, the proposal really consists of two ideas; that of an equilibrium, and that of a space into which equilibrium occurs. The concept of an equilibrium across an unspecialized endothelial wall rather than the alternative of a passage determined by endothelium of varying permeability characteristics is strengthened by the fact that little difference is found between the 6 hour and 24 hour tissue counts. If permeability were the key, a progressively wider disparity in the activity of different tissues would be expected to occur in the course of time; presumably, instead, a steady-state equilibrium is reached early and then maintained in dependency on blood levels which during the interval between 6 and 24 hours do not decrease enough to be reflected in CNS tissues. (An animal on which blood clearance of the albumin was measured showed values of 0.32 uc per ml. at 6 hours and 0.22 uc per ml. at 24 hours.)

The second part of the proposal, that equilibrium involves a pericapillary connective tissue space, has, in addition to the observations presented here, some support from a number of other studies in which connective tissue has been implicated to explain aspects of the blood-brain barrier. Thus it has been shown that acid dyes, which are as a group similar in size and distribution to albumin, exhibit an initially diffuse penetration into connective tissue before they are ultimately phagocytized as foreign particles (15); and indeed Lester King many years ago suggested a "specific affinity" of connective tissue for Trypan Blue, the acid dye with which he was working, in order to explain its CNS distribution (16). More recently, the experiments of Wislocki and others have demonstrated silver particles piled up along the borders of the perivascular connective tissue space, as well as scattered indiscriminately among the collagen fibers (8 to 9). Edström in a recent review has gone so far as to explain the entire barrier phenomenon on the paucity of CNS extra-cellular space, and makes a passing reference to the exceptional areas containing vessels with collagen sheaths.

While the sweep of this review may be open to question, it does appear from the other studies and the work presented here that at least with respect to a number of particles in the colloidal range, and albumin in particular, the perivascular connective tissue plays a major rôle. It is true that the silver aggregates seen by Wislocki resulted from the administration of ionic silver, but he speculates that the ions may be bound to serum protein in their transport through endothelium before being reduced to the visible metallic particle (18), and in view of the similarity of distribution to

albumin one might entertain the thought that serum albumin is the key protein involved.

That it is the connective tissue space which is important, rather than the collagen fiber, is made clear from a comparison between peripheral nerve and any of the 'special areas' inside the CNS. The proportion of collagen in peripheral nerve far exceeds that found in the special areas not because there is so much more connective tissue, but rather because the collagen in peripheral nerve connective tissue is so much more dense. One would therefore expect that if the collagen per se were the major factor, a correspondingly greater amount of albumin would be found in nerve; in fact, however, the value falls within the range of the 'special areas'. Another even more extreme test of this hypothesis can be made by comparing these areas with tendon. This was done on two animals not shown in the table, and the values obtained were actually lower than those of the 'special areas', a result certainly not expected with the operation of any collagen binding, but quite in keeping with the concept of available space.

In conclusion, then, the effect of a perivascular connective tissue space has been stressed in this paper, as an explanation for the distribution of serum albumin within the tissues of the central nervous system. The general concept is lent support by the observations of several other authors, although their interpretations have been modified. Thus the ideas of specific affinities, specialized endothelium, or even of a "succession of thresholds at different levels of which substances could be held up" (7) are felt to be unnecessary to explain the behavior of albumin, and very possibly other colloidal molecules as well. The so called exceptions to the blood-brain barrier are illusory; the barrier remains intact at the neuroglial cell layer.

SUMMARY

The distribution of blood-borne I^{131} labelled albumin within several tissues of the central nervous system in six cynomolgus monkeys is recorded. Assumptions concerning the homologous status, extravascular site, and intact label of the iodoalbumin are discussed. A uniformly low distribution among the purely neural tissues and cerebrospinal fluid was found, in contrast to tissues regarded as exceptions to the blood-brain barrier—area postrema, pineal body, and neurohypophysis. The characteristically large proportion of collagen that these tissues contain, compared with purely neural tissue, is placed on a quantitative basis and is seen roughly to parallel tissue albumin concentrations. An explanation is offered for this parallel, based on simple considerations of equilibrium and an available connective tissue space, rather than collagen fibers as such.

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POTASSIUM FLUX AND FURTHER OBSERVATIONS ON ALDOLASE FLUX IN DYSTROPHIC MOUSE MUSCLE[1]

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We reported previously that efflux of aldolase was accelerated from muscles of mice with hereditary muscular dystrophy (1) and that increased aldolase flux, if it occurs also in human muscular dystrophy, explains the high concentration of aldolase in serum, first observed by Sibley and Lehninger (2) and low concentration of aldolase in muscle of patients with muscular dystrophy, first reported by Dreyfus, Schapira and Schapira (3).

More recently Baker, Blahd and Hart (4) and Young, Young and Edelman (5) have determined that potassium concentration in muscle from mice with hereditary muscular dystrophy was less than that in muscle from their heterozygous and clinically normal litter-mates. Because we have noticed that many factors which altered permeability of muscle membrane to aldolase also altered permeability to potassium we suspected that potassium efflux might also be accelerated in muscle from these dystrophic mice. This proved to be true.

Besides reporting potassium efflux from dystrophic mouse muscle we are also reporting additional data on aldolase efflux. In our earlier experiments muscles were shaken in a Warburg respirometer. Because aldolase efflux from normal rat muscle is increased by even such simple manipulation as transferring the muscle from one flask to another (6), it occurred to us that mechanical agitation during shaking in the Warburg apparatus might have increased aldolase flux from both normal and dystrophic mouse muscle, thereby tending to reduce the difference between them. Accordingly we constructed respirometer chambers in which muscles could be kept at rest length. Under these circumstances, aldolase efflux was more definitely increased from dystrophic mouse muscle compared to that from muscle from normal litter-mates.

METHODS

Muscular dystrophy is inherited in mice as a recessive (7). Litter-mate pairs, one having muscular dystrophy, genetically dydy, the other clinically normal and genetically Dydy, were obtained from the Jackson Memorial Laboratory.

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Aldolase Efflux

Mice were 48 to 58 days old on the day of the experiment. They were anesthetized with sodium pentobarbital intraperitoneally and the extensor digitorum longus muscle was exposed and freed, its tendon tied with Nylon thread, the distance between the knots was measured and recorded as rest length, the muscle was removed and placed at rest length in a specially-constructed respirometer chamber. The chamber was made of Lucite, was a rectangular prism in shape, had a bent stainless steel pin fixed into one wall and a second pin on an adjustable screw fitted into the opposite wall. The threads holding muscle tendons were tied to the pins and the muscle pulled to rest length by means of the adjustable pin. To the roof of the chamber was cemented a standard-taper glass connector which fitted the Warburg manometer column through which the chamber was gassed. A gas outlet vent was also cemented to the chamber roof. The muscle was gassed continuously with 95 per cent O_2-5 per cent CO_2 during incubation at 37°. The bathing solution had the following composition (mM./l.): NaCl. 117; KCl, 3.5; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 28; glucose 11. Cytidine, 40 mg. per l., was also present. The pH was about 7.4. Incubation continued for two hours. Muscles were removed, blotted gently, threads and tendons snipped off, and and the muscles were weighed rapidly and then homogenized in distilled water at 4°C. Homogenates and solutions in respirometer vessels were analyzed promptly for aldolase by the method of Sibley and Lehninger (8). Aldolase in bathing solution (effluent aldolase) is expressed as per cent of total aldolase (effluent aldolase plus homogenate aldolase).

Potassium Efflux

Mice were 48 to 69 days old on the day of the experiment. A solution of $K^{42}Cl$ was injected intraperitoneally in a dose supplying approximately 25 μc per gram of body weight. Specific activity was about 25 to 100 μc. per mg. of K at the time of injection. Approximately two hours later, under sodium pentobarbital anesthesia, extensor digitorum longus muscle was removed as described above and tied at rest length in a chamber which formed the roof of a gas-flow Geiger counter. The muscle was gassed continuously (95% O_2-5% CO_2) and bathed by gravity flow with non-recirculating solution of the same composition as that used in experiments on aldolase flux except that no cytidine was present. Temperature was about 26°C. A more detailed description of the apparatus has appeared elsewhere (9). Potassium content of muscles was determined on acid digests of muscle by flame photometry.

RESULTS

Aldolase Efflux (Table I)

Eight pairs of mice were prepared for measurement of aldolase efflux. The muscle of one dystrophic mouse was torn during dissection and discarded. Aldolase efflux from one normal mouse differed from the normal mean by more than 16 standard deviations and probably was also torn although the accident was not recognized during the experiment. Data from this muscle are therefore excluded from calculations of mean efflux and of differences between pairs.

Aldolase concentration in the muscles was similar to that reported in

TABLE I

Aldolase Efflux from Dystrophic Mouse Muscle

Body Weight		Total Aldolase		Aldolase Efflux		
Control	Dystrophic	Control	Dystrophic	Control	Dystrophic	Difference
grams	grams	u/mg.	u/mg.	%/2 hr.	%/2 hr.	%/2 hr.
18	16	4.26	2.27	0.12	0.53	0.41
20	12	3.90	1.62	0.03	1.62	1.59
16	14	4.03	—	0.35	—	—
27	14	3.70	2.16	0.27	2.91	2.64
25	13	3.40	2.21	2.29	1.59	−0.71
20	13	3.55	2.41	0.18	6.13	5.95
28	16	3.51	2.43	0.40	1.15	0.75
Mean		3.76	2.18	0.22	2.32	2.23
± S.E.M.				0.05	0.75	0.91

Each line supplies data from one pair of mice. Total aldolase is in arbitrary units per mg. fresh wt. of muscle. Aldolase efflux is per cent of total aldolase diffusing out of muscle in 2 hrs. Aldolase efflux (2.29%/2 hrs.) from one control mouse excluded from calculation of mean.

1958, although aldolase in dystrophic muscle was somewhat less than in the earlier series, presumably because the average age of the mice was a few days greater than in the earlier series (i.e., the dystrophic process was more advanced). As was true in the earlier series and in the prior report by Weinstock, Epstein and Milhorat (10), aldolase concentration was clearly less in dystrophic than in normal mouse muscle.

Efflux of aldolase was reduced markedly in the present preparation compared to the earlier preparation in which muscles were subjected to mechanical agitation. Mean efflux from control muscles was about 2 per cent per 2 hr. in the earlier studies, but it was only one-tenth as great, 0.2 per cent per 2 hr. when muscles were held at rest length. The effect of the present technique on aldolase efflux from dystrophic muscle was less striking. In the earlier series, aldolase efflux from dystrophic muscle was 3.25 per cent per 2 hr. compared to 2.32 per cent per 2 hr. in the present series.

These data confirm our previous report that muscles from dystrophic mice were leakier with respect to aldolase than those of their normal littermates and demonstrate that the increased permeability of dystrophic muscle is even relatively greater than we had estimated previously. It is quite likely that mechanical agitation during shaking in the Warburg apparatus did increase permeability of both normal and dystrophic muscle to aldolase but, apparently, dystrophic muscle is already so permeable that the added effect of mechanical agitation was less destructive than it was in normal muscle.

Potassium Efflux

There is no doubt that K^{42} moved out of dystrophic mouse muscle much more rapidly than it moved out of normal mouse muscle (Fig. 1). In each of the seven pairs of muscles (control vs. dystrophic litter-mate) this was true. The difficulty comes in quantifying K efflux owing to the heterogeneity of muscle fiber size, particularly in dystrophic mice.

Complete formal treatment of the effect of heterogeneity of fiber diameter is rather complex and will not be undertaken here. Suffice it to say that in a collection of parallel cylinders of equal length, the decrease in radioactivity from every cylinder with time is proportional to $e^{-ct/r}$, where c is a constant and r is the radius of the cylinder. Therefore, the smaller the radius, the more rapidly will radioactivity decrease. The decrease in radio-

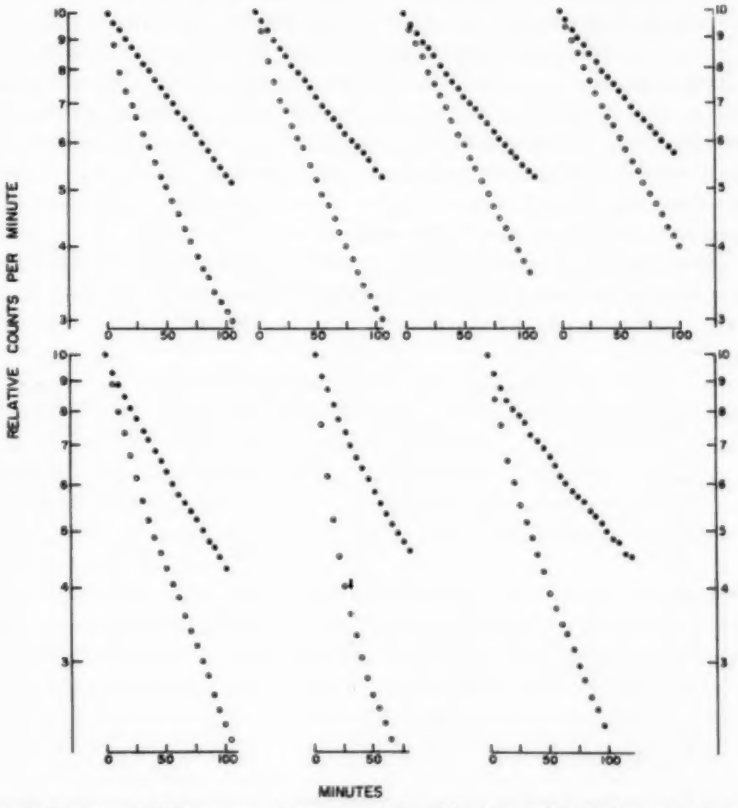

FIG. 1. Decrease in K^{42} content of mouse muscle with time. Solid circles, control mice; open circles, dystrophic mice. Counts per minute at zero time adjusted to same value.

activity from the collection of cylinders is the sum of all such terms in $e^{-ct/r}$ and therefore cannot, in general, be described by a single rate constant. The initial rate is determined by the narrowest cylinders; the final rate, by the widest cylinders. If the variation among cylinder radii is not very large, a plot of logarithm of counts per minute remaining in the muscle against time may very well produce a reasonably good straight line, even though there is in fact more than one rate constant. This was usually true for normal muscle. However, if there are many more narrow fibers in dystrophic muscle, the initial rate of decrease in radioactivity would be expected to exceed that found in normal muscle even if permeability per unit surface area was the same as in normal muscle. Furthermore, because dystrophic muscle does contain a mixture of unusually narrow fibers, normal fibers and swollen fibers, the distribution of fiber radius is probably flatter than the distribution in normals. This means that the curvature of a plot of logarithm of counts per minute against time should be more obvious than in normal muscles, as indeed it was. Nevertheless, it was always possible to find, after an initial brief rapid decrease in radioactivity, a well-sustained decrease which could be fitted to a single rate constant. Comparison of this rate constant with that of paired normal litter-mates appears in Table II. The rate constant was always higher in the dystrophic mice; that is, decrease in radioactivity was always faster in dystrophic mice.

Unfortunately, this analysis gives no information as to whether or not there was in fact greater permeability per unit surface area in dystrophic

TABLE II

K Efflux and Muscle K in Dystrophic Mouse Muscle

Body Weight		Muscle K		Rate Constant, K Efflux		
Control	Dystrophic	Control	Dystrophic	Control	Dystrophic	Difference
grams	grams	mM./kg.	mM./kg.	hr.$^{-1}$	hr.$^{-1}$	hr.$^{-1}$
19	14	115	88	0.26	0.62	0.36
20	14	91	85	0.27	0.45	0.18
20	12	102	104	0.27	0.58	0.31
18	12	124	95	0.28	0.49	0.21
22	16	103	88	0.32	0.59	0.27
25	13	102	93	0.39	0.67	0.28
16	11	93	?	0.41	0.67	0.26
Mean		104	92	0.31	0.58	0.27
± S. E. M.		4.1	2.5	0.02	0.03	0.02

Each line supplies data from one pair of mice. Muscle K is in mM. per kg. fresh wt. at time zero (i.e., before incubation). The datum marked ? was 50 mM./kg., so different from the rest that it is assumed to be in error and is excluded from calculation of the mean.

muscle. One could make this assessment only if there were exact knowledge of the distribution of fiber radii in both normal and dystrophic mice, but there is not. Even with such information, the assumption that permeability per unit surface area was independent of fiber radius in normal mice might not be justified. Indeed, if the ratio, permeability (per unit surface area) to radius were constant this would explain the fact that decrease of radioactivity from normal muscle seems to fit a first-order curve rather well despite the fact that there is a distribution of fiber radii.

Potassium Concentration

Baker, Blahd and Hart (4) and Young, Young and Edelman (5) found the concentration of potassium in dystrophic mouse muscle low both per unit wet weight and per unit fat-free dry weight. We confirm this observation, Table II, although the difference is less marked in our series, probably because we used younger (therefore less severely dystrophic) mice.

An important difference occurred during the two hours during which radioactivity was measured. The data on potassium concentration in Table II are those of the contralateral muscle which was frozen immediately after excision and held for analysis. After two hours in the chamber the experimental muscle was frozen. In muscles from normal mice, potassium concentration decreased during the two hours (compared to the contralateral muscle) by an average of 8 per cent. In muscles from dystrophic mice the decrease in potassium concentration was by 33 per cent. Decrease in potassium concentration, of course, can occur only if potassium efflux exceeds potassium influx.

In our experience with the same muscle in young rats under similar conditions there was no decrease in muscle potassium concentration during two hours in the bath and the rate constant for efflux was a good deal smaller (9). This difference raises the possibility that the control mice in these experiments, which were heterozygous Dydy, may not have been normal and that muscles from mice which are DyDy may prove to be less permeable to potassium.

Our immediate interest centers on the relative rate of potassium influx. Efflux of potassium is proportional to the internal potassium concentration. The constant of proportionality was 88 per cent larger in dystrophic muscle than in normal muscle and the potassium concentration was, initially, 12 per cent less. The ratio of efflux of potassium (dystrophic/control) was therefore $1.88 \times 0.88 = 1.65$, or potassium efflux was 65 per cent greater in the dystrophic mice.

From the equation, $[K^+_i]\ (t) = [K^+_i]\ (0)\ e^{-(k_i - k_0)t}$, where $[K^+_i]\ (t)$ is muscle potassium concentration at time t, k_i is rate constant for influx,

and k_0 is rate constant for efflux, and from the observed changes in $[K^+_i]$ at $t = 2$ hours and from the measured values for k_0, k_i can be calculated as 0.27 hr.$^{-1}$ for the control mice and 0.38 hr.$^{-1}$ for the dystrophic mice. The rate constant for potassium influx was therefore 41 per cent larger in dystrophic muscle than in control muscle. However, owing to the difference in initial concentration, the actual influx was only 24 per cent greater into dystrophic muscle than into control muscle.

Although these observations still do not let us quantify the effect of differences between distibutions of fiber radii, they do tell us that the observed differences in potassium efflux cannot be attributed solely to the presence of a relatively large population of narrow (and hypothetically normally permeable) fibers in dystrophic muscle because the effect of fiber radius applies with equal force to both influx and efflux. The greatest possible effect of a population of narrow, but normally permeable, fibers in dystrophic muscle is presumably manifest in the 41 per cent increase in rate constant for influx. This implies that there was a real change in permeability of the muscle membrane to potassium efflux by no less than 47 per cent ($= 88 - 41$) in dystrophic muscle.

Of course, it is fiction to speak of altered permeability in one direction. Presumably, permeability to inward movement of potassium also was increased, but most of the inward movement of potassium is driven by the electrical potential difference across the muscle membrane. Failure of influx to match efflux to such a great extent as seen here suggests that the resting membrane potential probably decreased during the two hours of observation in dystrophic muscle.

It is quite unlikely that such large differences between influx and efflux of potassium exist in the animal. In the normal mouse, *in vivo*, potassium influx and efflux must be equal, at least over some reasonable time interval, such as 24 hours, otherwise muscle potassium concentration would fluctuate more than present information suggests. On the other hand, in the dystrophic mouse, *in vivo*, potassium efflux must at sometime exceed influx, otherwise muscle potassium concentration could not have decreased from normal values. The difference between influx and efflux must be rather small *in vivo* since there was only a 12 per cent decrease in muscle potassium concentration in mice seven or eight weeks old, certainly incompatible with a net loss of 20 per cent per hour such as occurred during the two hours *in vitro*. Therefore, dystrophic muscles were less able to survive the *in vitro* experiment than control muscles. This may have occurred because they may be more fragile and more easily damaged by the manipulations or because they require certain substances absent from the bath but normally present in the mouse's extracellular fluid and because their

requirement for this substance (or these substances) is either unique compared to normal mice or quantitatively greater than in normal mice.

However, it is difficult to escape the conclusion that muscles of dystrophic mice are unusually leaky, evidently for potassium and aldolase, and one would expect them to prove leaky to a number of other substances.

SUMMARY

Drystrophic mouse muscle is more permeable to aldolase than muscle from clinically normal heterozygous litter-mates, even when aldolase flux is minimized by holding muscles at rest length.

Both potassium efflux and potassium influx are greater in dystrophic than in control muscle. The increase in potassium efflux cannot be attributed entirely to the probability that there may be a large population of narrow fibers in dystrophic muscle because the efflux greatly exceeded the influx, and the effect of fiber radius on flux affects influx and efflux equally.

In confirmation of others' observations, potassium concentration is less in dystrophic than in control muscle. This is probably the result of accelerated potassium efflux, although the fluxes measured *in vitro* must have been greater than those that one can reasonably expect to occur in the living mouse.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY
January 9, 1961

EPIDEMIOLOGIC INVESTIGATION OF SARCOIDOSIS

ALFRED A. BUCK

Recent epidemiologic investigations of sarcoidosis have disclosed new facts about the distribution of the disease. On the whole, these contributions have stimulated the future use of epidemiologic studies of this still enigmatic disease. In the present study an attempt was made to apply such methods to an investigation of selected and still unconfirmed features attributed to sarcoidosis, and to test, if possible, the validity of some of the results obtained by earlier investigators.

A case of sarcoidosis was defined as a patient with both clinical symptoms and histologic findings compatible with sarcoidosis, who was treated or at least examined once in the Moore Clinic of the Johns Hopkins Hospital, and whose residence was in Maryland. These patients, their spouses and their first-degree relatives were interviewed and skin tested with 5 TU PPD and *Candida albicans* antigen. From healthy family members a blood sample for paper electrophoresis was drawn at the time of reading the skin test. For each case a control subject, matched for sex, race and approximate age, was selected at random from the 634 outpatients registered in the files of the Moore Clinic. These controls, their spouses and their first-degree relatives were interviewed, examined and tested in the same manner as those of the sarcoidosis group. Altogether there were 376 individuals available for the study with 62 pairs of sarcoidosis cases and controls. Cases and controls were comparable with respect to the same mean age and family size, birth order, marital status and migratory trends. Likewise, the percentage of relatives who refused to participate in the study showed only slight differences.

A comparison of the frequency distribution of induration sizes resulting from tests with tuberculin and *C. albicans* antigen, as observed among 58 pairs of cases and their matched control partners, supported the hypothesis of a *general* suppression of delayed-type hypersensitivity reactions associated with sarcoidosis. On the other hand, a comparison of the results of skin tests with tuberculin and *C. albicans* between first-degree relatives of cases and controls did not show differences which could support the hypothesis of a familial, constitutionally determined suppression of delayed-type hypersensitivity reactions. A general factor of suppression of skin reactions could be computed for the 62 sarcoidosis cases, if the first-degree relatives of cases were used as a control group for a comparison of age-adjusted prevalence rates of reactors to tuberculin and *C. albicans* antigen.

An analysis of the results of serum protein fractions, determined by the use of paper electrophoresis, revealed significant differences of the variance of gamma-globulin levels between members of the same family and individuals of different families. A familial pattern of the gamma-globulin fraction could be computed for both the healthy first-degree relatives of the cases and of the controls. On the other hand, a comparison of the observed gamma-globulin levels of healthy relatives of cases with those of controls showed a significantly larger proportion of individuals among family members of cases with values exceeding 2.0 grams per cent of gamma-globulin. Similar results could be obtained for the family mean values. No significant differences between the two

groups of relatives could be detected for the albumin, alpha 1, alpha 2 and beta fractions of serum proteins.

Systematic screening of 62 families of sarcoidosis cases and controls resulted in the detection of 5 with multiple cases, whereas no case could be found among the similarly screened control families. The results seem to support the hypothesis of a familial aggregation of sarcoidosis, which was formed on the basis of case reports of 40 families with multiple cases published in the literature.

Among other results of the study, which were based on personal interviews, the predominance of rural birthplaces and rural residences among persons with sarcoidosis was confirmed. In addition, it was shown that persons with sarcoidosis had spent a larger proportion of their lifetime in rural places than did their control partners of approximately the same age.

A detailed description of the various results of the study will be published elsewhere.

TRANSMISSION OF AIRBORNE INFECTIONS

R. L. Riley

The mechanisms of transmission of airborne infection which have to be seriously considered are dust, respiratory droplets and droplet nuclei. These three mechanisms involve particles which differ in their aerodynamic behavior. Ordinary dust and coarse respiratory droplets settle while droplet nuclei, which are the dried residues of the finer respiratory droplets, have no appreciable tendency to settle. Because of this aerodynamic difference, microorganisms associated with droplet nuclei must be removed from room air by ventilation or by killing the organisms while suspended in the air. The term sanitary ventilation applies to removal of airborne organisms by either of these methods. Since sanitary ventilation removes infectious droplet nuclei but does not remove organisms borne on dust or droplets, the effectiveness of increased sanitary ventilation in stopping the spread of infection constitutes a selective test for infection which is transmitted by droplet nuclei. In three of the four studies of airborne infection to be discussed, this test is applied under widely varying circumstances. The first study provides more direct evidence for droplet nucleus transmission in the case of pulmonary tuberculosis.

At the Veterans Administration Hospital in Baltimore a colony of guinea pigs averaging 156 in number breathed the air vented from a six bed tuberculosis ward continuously for two years. Seventy-one guinea pigs became infected, an average of approximately 1 every 10 days. Since the guinea pigs breathed approximately 12,000 cubic feet of air in 10 days, one infectious dose of airborne tuberculosis was contained, on the average, in this volume of air. If the ventilation of the ward had been doubled, the infectious droplet nuclei would have been diluted in twice as much air and only half as many guinea pigs would have been infected. Chance of infection was thus inversely related to ward ventilation. A repetition of the experiment for a second two year period, using additional controls, has confirmed the results of the first two years.

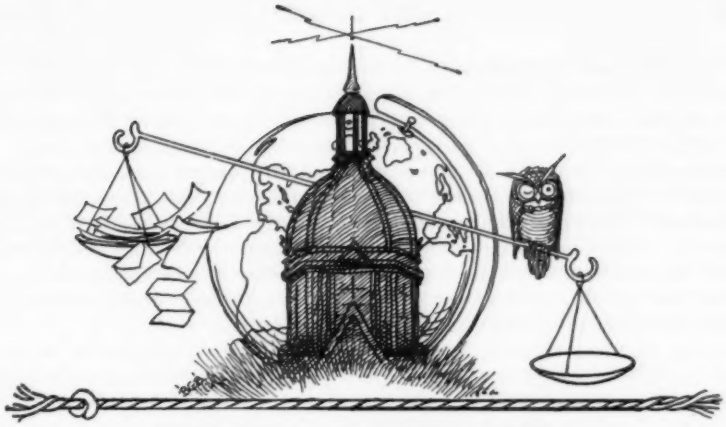
In 1941 Wells, Wells and Wilder studied an epidemic of measles among school children in Swarthmore and Germantown, Pennsylvania. The upper air of the primary classrooms was irradiated with ultraviolet light. An impressive degree of control was achieved. In the succeeding five years, the incidence of measles was consistently lower in three irradiated schools as compared with three unirradiated schools. Over this prolonged period the effective contact rate among the children in the unirradiated classes was .10. Among the children in the irradiated classes it was .03. This study has become controversial because other workers have not been able to get comparable results. In

view of the fact that children are in their classrooms less than 20 per cent of the time, classroom irradiation cannot succeed unless the chance of infection out of school is relatively slight, a circumstance which appears to have prevailed in Germantown and Swarthmore to a greater degree than in the communities where the results could not be duplicated. This classic study demonstrated an inverse relation between sanitary ventilation (achieved by ultraviolet irradiation) and the chance of catching measles.

Dr. Mildred W. Wells analyzed seasonal patterns of measles and chickenpox in relation to climate. In cold weather people close the windows and stay indoors, thus reducing sanitary ventilation per susceptible. Records of 20 years from states along the eastern seaboard show that the length of the season for measles and chickenpox is related to the length of the cold season. The results of this gigantic experiment of nature are consistent with an inverse relationship between sanitary ventilation per susceptible and chance of infection.

In 1957 and 1958 Dr. Ross McLean studied the incidence of Asian influenza and other acute respiratory infections at the Veterans Administration Hospital in Livermore, California. The upper air of all the rooms and corridors of one large hospital building was irradiated with ultraviolet. In the fall of 1957 there was an outbreak of non-specific acute upper respiratory infection and in January, 1958, there was an outbreak of serologically proven Asian influenza, both occurring among patients occupying the unirradiated hospital buildings. There were no outbreaks among the patients in the irradiated building, suggesting again an inverse relationship between sanitary ventilation per susceptible and chance of infection.

The results of these four diverse experiments converge on a single interpretation. Transmission of the types of infection considered can be interrupted by increasing sanitary ventilation, a method of control which is selective for droplet nuclei. Sanitary ventilation is thus a powerful tool with which to find out how different types of epidemic respiratory contagion are spread.



BOOK REVIEWS

Man and His Body. The Wonders of the Human Mechanism. By BENJAMIN F. MILLER AND RUTH GOODE. 375 pp., $5.95. Simon and Schuster, New York, N. Y., 1960.

This book is a carefully planned exposition of the functioning of the human body directed at that large group of readers, who, though they may not have yet received broad biological and chemical training, are interested in the mechanism of the body. Readers might well include such categories as the high school or college student who is considering a career in medicine or one of the medical sciences, the college biology student, or a graduate chemist who desires to extend his knowledge of the application of his field of special competence.

Separate chapters include essays on the function of the several organs of the body, the mechanisms of individual cells, and the coordinated functioning of the whole body, with reference to growth, endocrine control, reproduction, and the nervous system.

The volume has competent authorship. Dr. Miller is a physician with special training in physiology; he is now director of the May Institute for Medical Research of the Cincinnati Jewish Hospital and an associate professor at the University of Cincinnati College of Medicine. Mrs. Goode is a writer with experience as an author of several volumes portraying for the general reader the fields of medicine and psychiatry.

PALMER H. FUTCHER

Practical Clinical Management of Electrolyte Disorders. By WILLIAM J. GRACE. 126 pp., $4.95. Appleton-Century-Crofts, Inc., New York, N. Y., 1960.

Dr. Grace has achieved, eminently, his declared purpose of describing the management of electrolyte disorders with a simple presentation, clinically oriented.

Employing a terse style, generously interspersed with italics, capitalizations, summaries, outline forms, and even maxims, he has succeeded in dehydrating his material to its bare essence. There is nothing superfluous—it escapes, somehow, desiccation. He has skillfully maneuvered between Scylla and Charybdis. Numerous case reports, studded through the pages, provide texts for very simple, yet fundamental explanations of the fluid and electrolyte disorders involved.

There are ten chapters, all but three introduced by the phrase "What happens in",

an expression more apposite, possibly, to a series of a more fictional nature. These chapters are concerned with the problems encountered in gastric suction or vomiting and alkalosis, heart failure, chronic and acute renal disease, diabetes, magnesium deficiency and aldosteronism. Two chapters, "Replacement of electrolyte deficits", and "Mechanisms of electrolyte disturbances; control of hydrogen ion concentration" are described in a carefully methodical and elementary fashion that affords the reader the satisfaction of familiarity and anticipation. The Henderson-Hasselbalch equation is precisely explained with accompanying standard diagrams and chemical formulations.

For those interested in expanding this condensation of knowledge, there are many general and specific references, not only to classically comprehensive publications, but also to papers on current progress and development.

For the medical student or the interne to serve as a preliminary frame on which to build, for the practicing physician as a review and recall of fading basic knowledge, this monograph succeeds in fulfilling its design.

A novice boatman was lost on the bay, with a storm breaking. When he hailed a passing sloop for home port directions, and the veteran skipper advised, "West Northwest and one point North", the harassed amateur shouted beseechingly, "Don't get so technical, just point".

To those who can sympathize with the boatman, Dr. Grace's pointing will be appreciated.

MATTHEW DEBUSKEY

Pharmacology in Nursing, 8th edition. By ELSIE E. KRUG. 805 pp., $6.00. The C. V. Mosby Co., St. Louis, Mo., 1960.

The eighth edition of this textbook shows considerable revision bringing the latest and most used therapeutic agents to the attention of the nursing profession. The tables "Metric Doses and Apothecary Equivalents" on the inside of the front and back covers of the text are of considerable value and are not found in previous editions. The section at the back of the 7th edition entitled "General References" has been deleted and the references pertaining to the material discussed in each chapter are presented at the conclusion of the chapter according to specific topics.

Several other new features in this text contribute to the value of this book as a textbook for nursing students. The combining of the chapters, "The Orientation to Pharmacology" and "How to Study Pharmacology", into the first chapter results not only in a good introduction for the student, but tends to illustrate the importance of acquiring an adequate and usable knowledge of pharmacology. The inclusion of International and Canadian drug standards helps to broaden the scope of the book. Presenting the active constituents of plant drugs earlier tends to provide a more logical sequence for study; although any and all the chapters can be presented in whatever sequence desired by individual instructors.

Another new feature, which I found most valuable, is the diagraming of the structural formulas of several drugs. This, I feel, enables the student to see the basic relationship of new therapeutic agents having similar action. Having "side effects" and "toxic effects" discussed for all major dugs, rather than just for certain few drugs, enables the students to determine the nursing care of patients receiving these agents. The dividing of "Chemotherapeutic Agents" into two chapters—Systemic Anti-infectives and Local Anti-infectives—rather than discussing them in a single chapter is in keeping with the central theme of the book, presenting drugs according to their action on the systems of the body.

Miss Krug has been very successful with the eighth edition for it is presented in such a manner that it presents basic knowledge upon which the student can build continuously. I consider this an excellent textbook for basic nursing students.

BARBARA HIBYAN

Group Processes. Transactions of the Fifth Conference, October, 1958. Edited by BERTRAM SCHAFFNER. 196 pp., $4.50. The Josiah Macy, Jr. Foundation Publications, New York, N. Y., 1960.

This is the fifth annual multiprofessional conference in the Group Processes series, designed to stimulate research and exchange of ideas between biologists, physiologists, pediatricians, animal psychologists, sociologists, anthropologists and psychiatrists. There were twenty-five participants.

The Conference covered three areas. The section on Experimental Aspects of Pediatrics dealt with factors and mechanisms involved in homeostatic capacity of the organism in relation to the internal, physical, and emotional environment. The difficulties of obtaining clinical data and measurements on new-borns of physiological responses were described and related to the various instabilities and modifications in response to the same stimuli. There were a few comments about assorted aspects of mother-child relationships.

The section on The Analysis of Behavior in Terms of Control Systems describes an approach to the study of physiological homeostatic processes, involving different feedback mechanisms, by which problems and processes can be set up and then worked out by mathematical and logical processes. Although this is a complicated presentation, it can be followed with clarity.

The section on The Cult as a Condensed Social Process was based on anthropological observations on the Manus people of the Admiralty Islands in 1928 and again in 1953. The rise and fall of several cultist movements (semi-religious, semi-political), are described in detail, and with sufficient observational data to provide a picture of these people as they were first studied, and then after twenty-five years, when the effects of the white man's influences could be studied.

These conferences are undoubtedly more productive to their participants than they are to the readers of these reports. The difficult editing work is very well handled, despite the occasional obvious gaps in the continuity of the reporting. This book illustrates a cross-sectional limited sampling of three areas of investigational approach to behavioral problems, and provides a demonstration of interdisciplinary thinking and exchange in action.

THEODORE M. FELDBERG

Occupational Diseases and Industrial Medicine. By RUTHERFORD T. JOHNSTONE AND SEWARD E. MILLER. 482 pp., $12.00. W. B. Saunders Co., Philadelphia, Pa., 1960.

This book represents to some extent a revision of earlier texts by the senior author who has had wide experience as a consultant in industrial medicine and as clinical professor of medicine at the University of California. This new text has the advantages of the administrative, teaching and pathological experience of the junior author, who is director of the Institute of Industrial Health at the University of Michigan.

The practice of industrial medicine has been rapidly changing during recent years from the old type of surgical service for the treatment of occupational injuries to a medical service. In the modern concept of occupational medicine, the prevention and treatment of occupational diseases and other effects of the working environment are combined with a program for promotion of the worker's health, including his emotional

and mental health as well as his total physical well being. This modern concept of industrial medicine and the practical measures for carrying out such a program in industry form the basis of Part I of the book, "The Practice of Industrial Medicine." Compensation insurance and rehabilitation of the handicapped worker also are included, since a preventive medical program must consider the social and economic factors in illness.

Although 80 per cent of the text is devoted to the occupational diseases (Part II of the book), of necessity, many of the less important conditions could be covered only briefly. Other diseases of greater interest are discussed more fully with descriptions of common occupational exposures, pathology, symptoms, prevention and treatment. This type of organization leads to some duplication but makes for easy and quick reference use.

As an appendix a list of the 1960 "Threshold Limit Values" is reproduced. These figures represent the maximum concentrations of chemical contaminants in the air which are currently believed to be without adverse effects for daily exposure of industrial workers. A glossary of industrial terms also is included to assist the reader who is unfamiliar with industrial processes and materials.

The authors state that this book is not written for the experts in industrial medicine but chiefly for practitioners of general medicine who care for the needs of industrial workers and for students in occupational medicine. It is recommended for these groups and also for public health and industrial personnel who, although not specialists in this field, need to understand the basic principles of industrial medicine and occupational diseases. The chief shortcomings of the book stem from the necessity of condensing and simplifying a large and complex subject into one short volume which will be useful to the nonexpert. The authors have succeeded well in producing a clearly organized, readable and practical textbook which fills a definite need in the literature on this subject.

ANNA M. BAETJER

Demonstrations of Physical Signs in Clinical Surgery, 13th edition. By HAMILTON BAILEY. 928 pp., $14.50. John Wright & Sons, Bristol, England. The Williams & Wilkins Co., exclusive U. S. agents, Baltimore, Md., 1960.

This book is one of the most outstanding texts in the field of surgical physical diagnosis. The volume has increased in value and size with each edition. The new publications have added strength in the text and also the illustrations. To the reviewer's mind many of the pictures are so vivid that one glimpse makes a lasting impression. Composite-type illustrations and unusual originality are additional features. The scope of the volume extends beyond general surgery to the related specialities.

The book is particularly recommended to all medical students as a reference and to those associated with any aspect of surgery. It should be added that this edition must be regarded as a classic and would be a useful addition to the bookshelf of students of medicine at any level.

WILLIAM F. RIENHOFF, III

French's Index of Differential Diagnosis, 8th edition. Edited by ARTHUR DOUTHWAITE, 1111 pp., $24.00. The Williams & Wilkins Co., Baltimore, Md., 1960.

The first edition of this text was edited by Herbert French and appeared in 1912. Like Dr. Douthwaite, the Editor of the current edition, Dr. French was on the staff of Guy's Hospital in London.

In his preface to the first edition, Dr. French indicated that the book constituted an

index of symptoms, which are described in alphabetical order in the first 950 pages of the book. Simultaneously, the volume was to constitute a work on differential diagnosis, offering in the text discussion on the problem of distinguishing between the various diseases in which an individual symptom may be observed. This latter differential function is facilitated in the current edition by the allocation of the final 150 pages to a tabulation of diseases, with an associated listing of the symptoms occurring under each disease. To quote the first edition, "The guiding principle throughout has been to suppose that a particular symptom attracts special notice in a given case, and that the diagnosis has to be established by differentiating between the various diseases to which this symptom may be due".

The present edition is the work of Dr. Douthwaite and 19 other physicians and surgeons. The symptoms described ranged from "accoucheur's hand" through "weight, loss of". The volume contains in abbreviated form much material that is present in other monographs available to most physicians, such as text books on general medicine and surgery, on physical diagnosis, and on laboratory medicine. Some of the material might be considered extraneous, such as the section on the preparation of Benedict's and Fehling's solutions. There are no references to original articles. It is not unexpected that one can search unsuccessfully for information on some symptoms, such as the dry mouth and conjunctival symptoms of Sjogren's syndrome.

A consideration of other texts bearing on the same field of medicine as *French's Index* brings to mind Harvey and Bordley's *Differential Diagnosis*. The latter volume, while offering a listing of symptoms, is less an exhaustive index and more a readable and thoughtfully organized presentation of the systematic approach to medical diagnosis.

Whether *French's Index* is sufficiently useful to the individual physician to warrant its not inconsiderable cost is open to debate. The answer will depend in part on whether the physician finds useful an index in arriving at a diagnosis when faced with a difficult problem, and on the availability to him of other texts and publications which are more restricted in their content. That this text has proved valuable to physicians is suggested by the fact that it has been maintained for 50 years and is now in its 8th edition.

PALMER H. FUTCHER

Aids to Gynaecology, 12th edition. By W. R. WINTERTON. 214 pp., $3.00. Baillière, Tindall and Cox, London, 1960. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

This is a pocket-size volume which covers very basically the field of gynecology. It starts with the simple anatomy and physiology, then progresses through the various disease processes affecting the female pelvic organs and finally touches on the most common surgical procedures performed in gynecology.

The main value would seem to lie in its use by the medical student as a primer before he starts his gynecological studies. It fulfills this function admirably and the use of the British nomenclature and spelling are only mildly annoying. Most of the information is quite up-to-date; however, some areas, such as radiation therapy for benign disease, are stressed unduly. The lack of specificity and the dogmatic presentation of points upon which there are differences of opinion detract from the book's usefulness for other than an outline for review or study.

TIFFANY J. WILLIAMS

Modern Occupational Medicine, 2nd edition. By A. J. Fleming, C. A. D'Alonzo and J. A. Zapp. 587 pp., $12.00. Lea and Febiger, Philadelphia, Pa., 1960.

This book represents the collaborative effort of twenty-two authors, all of whom are associated full time with the E. I. du Pont de Nemours and Company. This second edition has the same general format as the earlier edition but is considerably longer because of new chapters on biostatistics and occupational chest diseases and fuller discussions on psychiatry and toxicology. In addition to these topics the book covers the organization of industrial medical departments; preventive medical services in industry; the effects of the physical environment, work and stress (rewritten in this edition); the more common acute nonoccupational poisons; and services allied to occupational medicine, such as sanitation, safety, surgical and nursing care, medicolegal aspects and rehabilitation of alcoholics. Since the book is a product from one of the large chemical companies, considerable emphasis is placed on chemical factors in the environment and practical measures for protection in such exposures.

The book presents the philosophy and the program of preventive industrial medicine as practiced by one of the most progressive industrial medical departments in this country. Much of the material is drawn from the personal experience of the authors. In addition, this text profits greatly from the contributions of the physiology and toxicology staff of the Haskell Laboratory who have presented much of their own original research data. The book is written for industrial physicians and others in the medical and allied professions who are concerned with the problems of industrial medicine. Because of the many authors, the book lacks the continuity and organization of a textbook, but it gains from the authoritative knowledge which the authors bring to their respective subjects.

Anna M. Baetjer

Annual Review of Medicine, Vol. 11, 1960. Edited by David A. Rytand. Associate Editor, William P. Creger. 453 pp., $7.00. Annual Reviews, Inc., Palo Alto, Cal.

This 400-page volume contains 23 articles. The content of the articles varies from pure physiology to clinical psychiatry. Each article is written by an authority in the field and although necessarily brief, is strengthened by the authors' ability to select the important aspects of the problem for discussion.

Perhaps the most valuable feature of the book is the extensive bibliography which follows many of the important articles. The individual articles do not represent a summary of all knowledge concerning the subject discussed, but do provide a good starting point with which to explore new knowledge in a variety of topics of current interest.

Julius R. Krevans

Diabetes. With a Chapter on Hypoglycemia. Edited by Robert H. Williams. 793 pp., $20.00. Paul B. Hoeber, Inc., Medical Division of Harper & Bros., New York, N. Y., 1960.

It is impossible for a single reviewer to do justice to this remarkable volume. With its fifty-four distinguished contributors, Dr. Williams has gone a good way towards selecting almost "everyone who is anyone" in the field of diabetes, though at times he must have had heart searchings on how to limit the number of authors.

The editor is to be commended for having included some six workers from overseas who are all well-known to their American colleagues and most of whom have worked for lengthy periods in this country. Though this cannot be regarded as an example of

proportional representation, it lends emphasis to the massive and outstanding contributions of the New World to the knowledge and understanding of diabetes.

Each of the forty-eight chapters is a complete work in itself, and the editor, as well as the contributors, has done well to avoid excessive overlap and repetition. The framework of the individual contributions varies, and clearly and wisely Dr. Williams has allowed much leeway to the members of his team. The references are carefully selected though, naturally enough, are not encyclopaedic.

In fact, this is no encyclopaedia of diabetes, and your reviewer has therefore found it possible to read it, during a somewhat prolonged period, from cover to cover. It embodies the personal contributions of the writers to diabetes; where scientific experiment and controlled study run short of relating a coherent story, philosophy and intelligent deductions take over. Fortunately, there is a minimum of wooliness of thought; as might be expected, this is found in the clinical chapters. There the need of the patient to receive some treatment regimen does not allow the physician to await the results of scientifically controlled trial and experiment.

There has been a flood of meetings and symposia in the general field of diabetes ever since carbutamide, the first established oral treatment for diabetes, was used in 1954 in Germany. Many of these meetings, usually sponsored by some drug manufacturer, presented the curious combination of scientific workers discussing their biochemical and physiological researches in metabolic areas linked to diabetes, and a rather more wooly clinical section which was meant to assess the new compound under scrutiny. Fortunately, the excellence of the first part of the program often overshadowed the second part.

Dr. Williams gives us another symposium, but what a breath of fresh air that the section on the oral compounds occupies but a scant twenty-seven pages out of 793. At last here we have a symposium which puts this area into perspective. All of us working in diabetes have learned much since 1954, and there is not a little some of us might wish to forget. Dr. Williams' brilliant symposium has an air of independence and scholarship about it, that makes us look forward with renewed hope to the work that must be done in the 60's to answer the many questions, ugly and otherwise, that have cropped up in the 50's. This volume is cheap at $20.00. May it see many editions. Fama Semper Vivat.

FREDERICK WOLFF

BOOKS RECEIVED FOR REVIEW

Blood Diseases of Infancy and Childhood. By CARL H. SMITH. 572 pp., $17.00. The C. V. Mosby Co., St. Louis, Mo., 1960.

The Central Nervous System and Behavior. Transactions of the Third Conference, February, 1960. Edited by MARY A. B. BRAZIER. Sponsored by The Josiah Macy, Jr. Foundation and The National Science Foundation. 475 pp., $7.50. The Josiah Macy, Jr. Foundation, New York, N. Y., 1960.

Clinical Applications of Cardiopulmonary Physiology. By M. HENRY WILLIAMS, JR. 233 pp., $7.50. Paul B. Hoeber, Inc., Medical Division of Harper & Bros., New York, N. Y., 1960.

Clinical Orthopaedics, No. 18. Internal Derangement of the Knee Joint. Edited by ANTHONY F. DEPALMA. 293 pp., $7.00 single number. J. B. Lippincott Co., Philadelphia, Pa., 1960.

Congenital Malformations of the Heart, revised edition. Vol. I, General Considerations. By HELEN B. TAUSSIG. 204 pp., $4.75. Published for The Commonwealth Fund by Harvard University Press, Cambridge, Mass., 1960.

Importance of the Vitreous Body in Retina Surgery with Special Emphasis on Reoperations. Edited by CHARLES L. SCHEPENS. 226 pp., $15.00. The C. V. Mosby Co., St. Louis, Mo., 1960.

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THE FRACTIONATION OF SEROMUCOIDS FROM HUMAN SERUM[1]

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A number of chemical investigations has been carried out on the seromucoid fraction of human serum (1). Although evidence has accumulated that this fraction is not homogeneous, no method has yet been reported which reproducibly or quantitatively is able to fractionate the serum mucoid fraction into distinct fractions (1). The method developed in this laboratory using Dowex 50 resins for fractionating seromucoids of human serum has not been found to be reproducible (2). We wish to retract all data mentioned in this communication (2) which were based on this method. Winzler using 500 ml. of sera and an electrophoretic technique has presented evidence that there are three components making up the serum mucoid fraction in human serum (3). One of these components, which has been termed orosomucoid made up approximately 60 per cent of the serum mucoid fraction (1).

In this paper, we wish to describe a chromatographic method using DEAE cellulose (4) which fractionates seromucoids into five fractions in a reproducible and quantitative manner. This method has been applied to the seromucoid fraction of seventy-five "normal" individuals. It will be shown that all these individuals have a fairly consistent seromucoid pattern.

METHODS

DEAE Cellulose

For the experiments described in the paper, cellulose obtained from Brown and Company, Berlin, New Hampshire was employed. The method used to wash the cellulose is given in detail as it was found that careful attention to all the details discussed below were necessary in order to have cellulose which did not give optical density reading of over 0.010 when blank columns were eluted with the eluents used on separating the seromucoid fractions. During the course of this work, five different lots of DEAE cellulose have been used and all have given similar results.

The cellulose is washed in jars, having a rectangular cross-section, which have a

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diameter of 12 cm. and are 20 cm. high. One hundred grams of cellulose is washed at one time. Three liters of 0.85 per cent saline are added and the mixture is stirred thoroughly for 10 minutes and then put in a refrigerator (4°C. to 6°C.) overnight. Most of the supernatant fluid is carefully siphoned off the next morning. The remaining fluid is removed in the following manner.

A nylon cloth, approximately 45 cm. square which has been thoroughly soaked and washed in 0.85 per cent saline is used to line a Buchner filter which is 25 cm. in diameter, and 8 cm. deep. The Buchner funnel is attached to a 4 liter suction flask. The cellulose is poured onto the nylon cloth and the remaining saline removed by suction.[2] The cellulose "cake" is then returned to a clean jar and 3 liters of 0.85 per cent saline immediately added and thoroughly stirred to a homogeneous mixture. The washing and separation is repeated 5 more times with 0.85 per cent saline. After the last wash with saline, 3 liters of 0.5 N NaOH is added and the cellulose "cake" is stirred thoroughly and constantly for 15 minutes. The 0.5 N NaOH is removed by suction as described above after which the cake is mixed with 3 liters of 95 per cent ethyl alcohol. This will cause the cellulose to "cake" so the mixture must be stirred vigorously for 15 minutes. The alcohol is removed by suction and the cellulose washed two times in 3 liters of 0.01 N NaCl. Each "wash" is stirred vigorously for 5 minutes. The cellulose "cake" is suspended in 3 liters of 0.01 N NaCl and adjusted to between pH 7 and 8 with 0.5 N HCl. Using suction, the fluid is siphoned off and the cellulose "cake" suspended in 3 liters of 0.5 N HCl and stirred thoroughly for 15 minutes. The acid is filtered off, and 3 liters of 95 per cent alcohol is added and the mixture stirred thoroughly for 15 minutes. After filtering off the alcohol, the cellulose is washed twice in 0.01 M NaCl and once in 0.5 N NaOH as described above. After filtering off the alkali, the "cake" is washed four times in 0.01 M NaCl and then three times in 0.001 M NaCl, using 3 liters for each "wash" and stirring each suspension for 10 minutes. The cellulose is left in the last 0.001 M NaCl "wash" and the pH adjusted to 7.5. The jar is covered and placed in the cold room (4°C. to 6°C.). The cellulose should stand 72 hours before being used and should occasionally be stirred. The pH of the cellulose mixture should always be determined the day before the columns are to be made up and readjusted to pH 7.5 if the pH has changed.

Preparation of Seromucoid Extract

In preparing the seromucoid extract for chromatographic separation, a modification of the Winzler method was used. As Winzler pointed out (1), there are a number of variables in this method which affect the yield of seromucoid. The method worked out in this laboratory which gave the best yields of seromucoid using the criteria of Winzler (1), and was most reproducible, is described below.

The fractionated method as described employs 30.0 ml. of serum. One person should make up only two preparations at one time in order to keep within the specified times employed in preparing the seromucoid fraction.

All procedures, except where noted, are carried out at 4°C. to 6°C. It is important that all solutions and receptacles used in preparing the seromucoid fraction be thoroughly equilibrated at this temperature before starting.

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[2] This method is used to remove all the fluids used in preparing the cellulose for chromatography.

Steps

1. Thirty ml. of serum is mixed in a 600 ml. beaker with 15.0 ml. of distilled water.

2. Thirty ml. of the perchloric acid solution (0.75 M) is added dropwise from a 10 ml. pipette at a continuous rate while the beaker is being swirled in a circular motion. The addition of the acid should be completed in about 90 seconds. As the acid is added to the serum a white precipitate in the form of clumps tends to occur. The beaker should be swirled at a sufficient rate to suppress the tendency for these clumps to form. After the addition of the acid, the beaker containing the mixture is left standing in a cracked ice bath for 3 minutes.

3. The mixture from 2 is then poured into a 250 ml. glass centrifuge bottle and centrifuged at 2000 $\times$ *g* at 4°C. to 6°C. for 10 minutes. After centrifuging, the supernatant fluid containing the seromucoids is poured into a 600 ml. beaker.

4. Fifteen ml. of 0.2 N KOH is quickly pipetted into the supernatant fluid making sure that the beaker is swirled vigorously in order to have the alkali diluted quickly as it mixes with the seromucoid solution. This mixture is then adjusted to pH 7.2 with 4 M KOH and left at 4.5°C. for 30 minutes so that the potassium perchlorate will crystallize out.

5. The supernatant fluid is poured off into a beaker and is ready for dialysis. The solution is dialyzed in dialysis tubing which has an inflated diameter of 27/32 inches and a flat width of approximately 1 5/16 inches. The tubing, obtained from the Arthur H. Thomas Company, Philadelphia, is cut into 12 inch strips and washed inside and out with distilled water ten to twelve times and stored at 4.5°C. in a large volume of distilled water.

6. The seromucoid preparation is poured into a piece of dialysis tubing. An air space of about 3 cm. is left in the dialysis tubing containing the seromucoid mixture. The top of the dialysis bag is securely tied to a 10 ml. pipette and the pipette immersed in 4 liters of distilled water.

7. The flask containing the preparation to the dialyzed is put on a magnetic stirrer at 4.5°C. and the water stirred vigorously using a magnet (Teflon).

8. After *15* hours of dialysis, the seromucoid mixture is removed, centrifuged at room temperatures for 5 minutes at 3000 $\times$ *g*, and the supernatant fluid is brought to room temperature quickly by immersing a flask containing the mixture in a water bath at room temperature (21°C. to 24°C.) and swirling the flask vigorously. The pH is adjusted to 7.2 with 0.03 N NaOH and the optical density (O.D.) at 280 mu is determined. It is now ready to put onto the column.

Preparation of Column

The washed cellulose described above is pipetted down the wall of the chromatographic tube using the large end of a 10 ml. pipette. The tube used for chromatography is made of 18 mm. standard wall pyrex which has a diameter of 15 mm. and is 20 cm. long with a drawn out tip. The small end is packed with 2 cm. of glass wool. The cellulose is pulled down the tube with suction using a vacuum of 10 inches of mercury until a cellulose bed of 4 cm. has accumulated. The column should not be left without at least 2 cm. of fluid covering the cellulose during the preparation of the column. The prepared column is stoppered and left at 4.5°C. overnight.

At the time that the seromucoid preparation is removed from the dialysis tubing, the columns should be removed from the cold room and equilibrated for two hours

at room temperature with 0.001 M NaCl which is also at room temperature. Approximately one drop a second of the salt solution should run through the column during its two hours equilibration period.[3] About 300.0 ml. of .001 M NaCl should drip through the column.

The seromucoid preparation is now ready to be put on the column.

The column and aspirator bottle containing the seromucoid preparation are attached to a Technicon time/flow fraction collector. The aspirator bottle is suspended over the column and attached to it by tygon tubing. The seromucoid preparation is dripped onto the column at a rate of 2.0 ml. per minute. Between 14 to 15 O.D. units should be put onto the column. The O.D. units are determined by taking the reading of the seromucoid preparation at 280 mu and multiplying this by the total volume of the solution that is dripped onto the column. After adding the required O.D. units, the aspirator bottle and tubing are rinsed out thoroughly with 0.001 M NaCl and then filled with 100 ml. of 0.001 M NaCl. When no fluid is dripping onto the column, the column should be stoppered, and a 2 cm. head of liquid maintained above the top layer of cellulose. Eight tubes of 0.001 M NaCl are collected using a flow rate of 5 ml. per 10 minute period. After the eight tubes have been collected, the aspirator bottle and tubing are thoroughly rinsed with 0.02 M NaCl and the bottle filled with 800 ml. of 0.02 M NaCl. The aspirator bottle is then connected to the column and fifty tubes are collected using the same flow rate as described above. Using the same technique the eluent is switched to 0.08 M NaCl and fifteen tubes are collected. The eluent is then switched to 0.135 M NaCl and the fraction is the next twenty-five tubes collected. The eluent is switched to 0.35 M NaCl using the technique described above and the last fraction is collected. The column can be shut off after fifteen additional tubes are collected.

The tubes containing 5.0 ml. of eluent are collected and all tubes are read in a Beckman spectrophotometer at 280 mu. While the tubes are being read at 280 mu, a standard containing 100γ of bovine serum albumin per ml. is read every twenty-five tubes to serve as a check on the spectrophotometer. All the Beckman spectrophotometers gave readings of 0.054 to 0.055 with 100γ per ml. of bovine serum albumin.

Chemical Tests

The tyrosine, hexose, and hexosamine content of each component were determined as described by Winzler (1). The tyrosine test is based on the color developed with the Folin-Ciocalteu phenol reagent in the presence of sodium carbonate, with the sample being read at 660 mu. The hexose test is based on the color developing in the presence of orcinol and sulfuric acid and reading the sample at 540 mu. The hexosamine test is based on hydrolyzing the sample in strong HCl adding an acetylacetone solution, reheating, and then adding p-dimethyl-aminobenzaldehyde solution containing strong acid and alcohol. The color of the sample is read at 535 mu. Protein was determined by the color developed on the addition of Folin-Ciocalteu phenol reagent in the presence of an alkaline copper solution and sodium tartrate. The samples are read at a wave length of 660 mu. Table I shows the standard deviations of the various methods. The determinations were carried out at weekly intervals for each test. At least two dilutions of each standard were always included in each test for all the chemical determinations of the various fractions.

[3] All solutions that are dripped onto the column are put into one liter aspirator bottles with tubular outlets, "pyrex" brand glass.

TABLE I

Reproducibility of Various Chemical Tests

	Number Determinations	Beckman Spectrophotometer Readings	
		Average	S.D.
γ of Tyrosine in standard solution			
8	24	0.096	0.00
16	24	0.197	0.004
32	6	0.408	0.008
γ of Hexose			
50	24	0.091	0.003
100	24	0.184	0.009
200	6	0.366	0.011
γ of Hexosamine			
15	24	0.141	0.004
30	24	0.271	0.009
45	6	0.425	0.010
γ of Bovine serum albumin			
50	24	0.029	0.002
100	24	0.059	0.003
300	6	0.151	0.006

The Analysis of the Carbohydrate Content of the Various Fractions by Paper Partition Chromatography

The following procedure was used in analyzing various fractions for various carbohydrates. The chromatograms were Whatman #1, 9 by 12 inch sheets of filter paper that were pinned together into the shape of a cylinder and run by the ascending technique. Approximately three milligrams of each seromucoid fraction studied was dialyzed against a large volume of water at 4°C. to 6°C. to remove the salts that were present. The fraction was then hydrolyzed in 0.01 N HCl at 100°C. for 60 minutes. This acid concentration and length of hydrolysis time was chosen to permit the release of any sialic acid and fucose with a minimal amount of destruction of the silalic acid (5). The sialic acid and fucose released by hydrolysis were then removed from the bulk of the seromucoid by dialysis and the dialyzing fluids from the given fraction were pooled and evaporated to dryness. The dry powder containing the sialic acid and fucose were dissolved in 20 to 30 lambda of water. The other sugars, glucosamine, galactosamine, mannose, and galactose were released from the non-dialyzable core of each fraction by hydrolysis in 3 N HCl at 100°C. for 4 hours. The hydrolysate was evaporated to dryness and dissolved in distilled water as described above. The hydrolysates were applied to the chromatograms as 2 to 4 millimeter spots, 2 centimeters from the lower end of the cylinder. The chromatogram was developed with a 10:4:3 mixture of ethyl acetate, pyridine and water (5) or a 1:2:1 mixture of n-butanol, n-propanol and water (6). The position of the sugars was visualized with $AgNO_3$ in acetone (7) as described by Hough (8). It should be pointed out that the actual carbohydrate composition must await the actual isolating and characterization of the sugars in

each fraction, since the reduction of $AgNO_3$ is too non-specific a developing agent to be used for a specific test for carbohydrates. However, as will be shown later in this paper, it does serve a useful purpose in this investigation by giving additional evidence that the fractions separated by the method reported in this paper differ chemically from each other and are thus distinct molecular entities.

Collection of Sera

Sera samples were collected from individuals who had no known diseases. This history was obtained by asking the volunteers whether they had had any chronic diseases in the past or whether they were ill at the present time. The blood was allowed to clot for one hour at room temperature at which time it was put into the refrigerator (4°C. to 6°C.). The next morning, the serum was collected from the individual specimens and stored at −20°C. in screw cap vials.

The seromucoid fraction after dialysis of all individuals described in this paper had O.D. readings varying between 0.240 to 0.320 at 280 mu when read in the Beckman spectrophotometer.

Procedure for Determining the Number of Units of Seromucoid in Each Fraction

In determining the concentration of seromucoid found in each fraction, the following standard chromatographic procedure was followed. The amount of seromucoid found in each fraction was expressed in terms of O.D. "units." The number of units eluted in a particular fraction was determined by taking all the tubes which gave O.D. readings higher than those observed when the same eluent was dripped through a column to which no seromucoid had been added. In this system, the background reading for any of the eluents used varied between 0.000 and 0.010 when read at 280 mu. Therefore, when an eluent was dripped through the column to which seromucoid had been added, the number of units in a particular fraction was determined by taking all tubes that read over 0.010 at 280 mu., after subtracting 0.010 from each tube, and multiplying these readings by the measured volume in all such tubes. These figures were then added up to give the total number of units eluted under the experimental conditions.

RESULTS

Methods of Extracting Seromucoids from Serum

In preparing the seromucoid fraction for chromatographic separation, a method had to be developed for extracting seromucoids from human serum. The reproducibility of the method based on eight determinations of aliquots of the same serum prepared at weekly intervals for eight weeks is shown in Table II.[4]

The procedure described under Methods was compared to Winzler's method (1) for preparing the seromucoid fraction. In the latter procedure,

[4] In all procedures for determining the reproducibility of the various parts of the method, the samples were coded by number so that the laboratory operators did not know the material being analyzed.

TABLE II

*Reproducibility of Preparing the Seromucoid Fraction from Human Serum**

Week	Absorbance Reading at 280 mμ
1	0.264
2	0.280
3	0.273
4	0.268
5	0.281
6	0.268
7	0.272
8	0.283
Mean	0.274
S.D.	0.007

* Seromucoid fractions were prepared from aliquots of the same serum sample which were frozen at −20°C. An aliquot was thawed once a week for 8 weeks and the seromucoid fraction prepared and its seromucoid content determined by reading the absorbance of the dialyzed solution at 280 mu.

filtration and precipitation with phosphotungstic acid is used instead of centrifugation and dialysis as described in our method. Table III shows that both methods agree very well as to the quantity of seromucoid recovered.

In our method, the quantitative recovery of seromucoid from serum was approached in the following manner. The seromucoid fraction was prepared by the addition of perchloric acid which precipitates the "non-

TABLE III

Comparison of the Seromucoid Content of Serum as Determined by Phosphotungstic Acid Precipitation and by Direct Determination After Dialysis

Experiment Number	Tyrosine/ml. as determined by:		Difference (1) − (2)	Hexose/ml. as determined by:		Difference (1) − (2)
	(1) Direct	(2) Phospho-tungstic Acid Precipitation		(1) Direct	(2) Phospho-tungstic Acid Precipitation	
1	52.1	53.1	−1.0	164.2	168.1	−3.9
2	96.2	106.2	−10.0	319.1	339.2	−20.1
3	39.1	37.3	+1.8	126.3	124.8	+1.5
4	53.8	55.2	−1.4	189.4	201.5	−11.1
5	49.1	47.8	+1.3	161.6	158.1	+3.5
6	45.4	47.2	−1.8	142.4	151.2	−8.8
7	56.3	49.5	+6.8	201.8	188.7	+13.1
8	48.5	52.4	−3.9	172.6	181.2	−9.6
9	84.2	79.3	+4.9	283.8	269.2	+14.6
	Total Difference		−3.3			−10.8
	Average Difference		−0.3			−1.2

TABLE IV

Effect of Repeated Extractions on the Apparent Total Yield of Serum Mucoids

Experiment Number	Number of Extractions of Precipitate with 0.3 M Perchloric Acid	γ/ml. of serum as Tyrosine	γ/ml. of serum as Hexose	Ratio $\frac{\text{Hexose}}{\text{Tyrosine}}$
1	Original supernatant	58.2	186.3	3.4
	Perchloric acid filtrate			
	First extraction	6.4	13.1	2.0
	Second extraction	2.7	4.1	1.5
	Third extraction	1.3	2.3	1.7
	Fourth extraction	1.2	1.8	1.5
2	Original supernatant	63.2	216.4	3.4
	Perchloric acid filtrate			
	First extraction	6.8	14.2	2.1
	Second extraction	2.1	3.9	1.9
	Third extraction	1.4	2.4	1.7
	Fourth extraction	1.3	1.9	1.4

seromucoid" materials. This precipitate was re-extracted a second time. This procedure was repeated two more times. Each of the supernatant fluids was analyzed for the "seromucoid" content as determined by tyrosine and hexose tests. From Table IV, it is apparent that the extractions yielded material which does not have the hexose to tyrosine ratios of seromucoid which should be around 3.5 (1).

Separation of Seromucoid Fraction into Distinct Components by Chromatography on DEAE Cellulose

Figure 1, shows the separation of the seromucoid fraction into five fractions employing the procedure described under Methods. The numbers on the graph refer to molar concentration of NaCl and the arrows indicate the time the noted eluent was added. Every tube was read in the Beckman spectrophotometer at 280 mu. The circles on the different peaks indicate the reading of the particular tubes at 280 mu in the spectrophotometer. Although no circles are shown in Figure 1 for the readings between the peaks, all tubes were read and the readings were below 0.010 which is the background reading.

Attempts to fractionate the seromucoid fraction by gradient elution using a continuous increase in ionic strength of sodium chloride rather than the stepwise elution method described above gave two or three fractions under the conditions of the experiment. In our hands, the stepwise elution scheme is a much more sensitive system for fractionating seromucoids. It might be argued that the fractions removed by the high concentrations of sodium chloride are really not different molecular entities than those removed by the low concentrations of sodium chloride

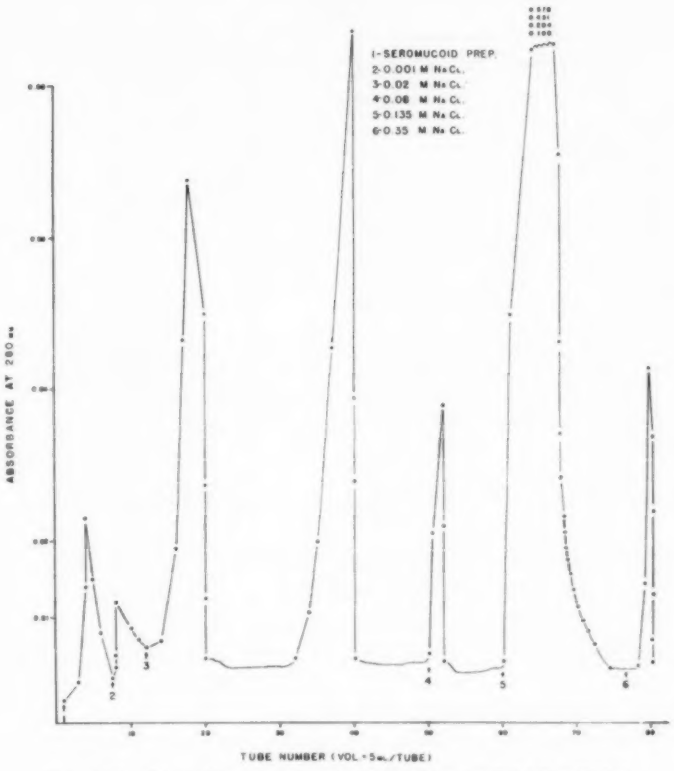

FIG. 1. Fractionation of seromucoid fraction on DEAE cellulose.

and that they are being eluted by a frontal displacement rather than a true partition elution. If this was so, a great deal of the fractions eluted by 0.135 M and 0.35 M of sodium chloride should be eluted by the low salt concentrations when these fractions are rechromatographed through the entire fractionation scheme. Such was not found to be the case as shown below. Furthermore, the difference in chemical ratios of the various fractions involving their hexose, hexosamine, tyrosine, and protein content and the differences in the carbohydrate content discussed later in this paper also strongly suggest that the five fractions are different molecular entities.

It should also be noted that two fractions are eluted with 0.02 M sodium chloride. It is possible that under these conditions a gradient elution has taken place.

Table V shows the reproducibility of the method when aliquots of the

TABLE V

*Test of Reproducibility of Chromatographic Fraction Method for Seromucoids When Aliquots of the Same Serum Sample are Fractionated on Different Columns at the Same Time**

Aliquot Number	Fraction #1	Fraction #2	Fraction #3	Fraction #4	Fraction #5
1	1.38	1.74	0.84	8.22	1.67
2	1.22	1.66	0.67	8.81	1.36
3	1.42	1.64	0.68	8.92	1.39
4	1.35	1.82	0.61	8.13	1.24
5	1.63	1.58	0.82	8.85	1.62
6	1.27	1.61	0.73	8.92	1.58
Mean	1.36	1.66	0.78	8.64	1.48
S.D.	0.132	0.083	0.082	0.333	0.156

* All values are given in total units recovered in each fraction. Columns 4, 5, and 6 were operated by 2 individuals different from those who fractionated the preparations on columns 1, 2, and 3. Fifteen units were put onto each column. The number of units found in each fraction in the above Table and subsequent Tables were calculated in the manner described under Methods.

same serum samples are fractionated on six different columns at the same time.

Table VI shows the reproducibility of the method when the aliquots of the same serum sample are fractionated at weekly intervals. The reproducibility is approximately the same in these two different tests and for chromatographic procedure is quite satisfactory. Approximately 85 per cent of the total amount of seromucoid absorbed onto the column can be recovered by this method (cf. Table VII).

Table VII indicates the variation found in the seromucoid pattern when

TABLE VI

*Reproducibility of the Fractionation Method as Determined by the Chromatographic Separation of Aliquots of the Same Serum Fractionated at Weekly Intervals for Eight Weeks**

Week	Fraction #1	Fraction #2	Fraction #3	Fraction #4	Fraction #5
1	1.13	1.52	0.68	7.8	2.13
2	1.36	1.54	0.81	7.2	1.64
3	1.42	1.31	0.72	7.9	1.97
4	1.18	1.62	0.62	7.2	1.74
5	1.25	1.21	0.71	7.8	2.25
6	1.45	1.28	0.79	7.9	1.78
7	1.18	1.61	0.83	8.1	1.91
8	1.09	1.48	0.85	7.2	2.31
Mean	1.26	1.44	0.75	7.5	1.97
S.D.	0.128	0.148	0.076	0.374	0.226

* With another serum the standard deviation was approximately twice that shown in Tables V and VI. Values under various fractions refer to total number of units recovered in each of the 5 fractions.

TABLE VII

*Concentration of the Various Seromucoid Fractions Found in Sera of Fifteen "Normal" Individuals**

Name	Col. No.	Age	Sex	Fraction #1		Fraction #2		Fraction #3		Fraction #4		Fraction #5		Total Units Recovered	Per Cent of Total Units Recovered
				Units Recovered	% of Total Units	Units Recovered	% of Total Units	Units Recovered	% of Total Units	Units Recovered	% of Total Units	Units Recovered	% of Total Units		
E.H.	1	49	M	1.2	7.8	1.7	11.1	1.0	6.5	7.4	48.7	1.4	9.2	12.7	85.3
E.S.	2	36	F	1.4	9.5	1.6	10.9	0.9	6.1	6.4	43.5	1.4	9.5	11.7	79.5
J.F.	3	45	M	1.1	7.2	1.3	8.5	0.9	5.9	7.8	51.6	1.2	7.9	12.3	81.1
S.T.	4	61	M	1.2	8.7	1.2	8.7	0.6	4.0	8.5	57.0	1.5	10.1	13.0	88.4
B.W.	5	72	M	1.1	7.2	1.5	9.8	1.3	8.5	6.9	45.3	1.3	8.5	12.1	79.3
N.M.	6	39	M	1.3	8.7	2.1	14.1	0.7	4.7	7.7	52.0	1.1	7.4	12.9	86.9
R.M.	7	41	M	1.2	7.9	1.4	9.2	1.2	7.9	5.9	39.0	2.3	15.2	11.0	79.2
K.S.	8	35	M	1.4	9.3	1.0	6.7	0.6	4.0	7.9	53.0	1.4	9.3	12.3	82.3
A.B.	9	38	M	0.9	6.1	0.8	5.4	0.8	5.4	8.8	59.4	1.8	12.3	13.1	88.6
E.M.	10	69	M	1.1	7.3	1.5	10.1	0.7	4.6	8.1	54.3	1.2	8.7	12.6	84.9
G.W.	11	45	F	1.4	9.3	1.8	12.0	0.7	4.6	6.9	46.0	2.5	16.6	13.3	88.5
G.L.	12	58	F	1.2	7.9	1.5	10.0	0.4	2.6	8.1	53.3	1.9	12.6	13.1	86.4
P.B.	13	47	M	1.3	8.5	1.8	11.8	0.8	5.2	5.3	34.9	2.8	11.1	12.0	61.5
P.R.	14	38	M	0.8	5.4	0.9	6.1	0.6	4.1	8.9	60.1	2.1	14.1	13.3	89.7
E.R.	15	42	M	1.1	7.2	1.3	8.5	0.7	4.6	7.9	52.3	1.9	12.5	12.9	85.1
Mean				1.1	7.8	1.4	9.5	0.73	5.2	7.5	50.0	1.6	11.0	12.5	83.1
S.D.				0.168		0.111		0.975		1.01		0.582		0.61	

* Between 14.7 and 15.2 units were added to the columns for each individual. The serum of P.B. was fractionated a second time and again a low recovery was found (66 per cent).

the sera of young and old "normal" adults were fractionated. It will be noted that all sera showed five components, although individuals showed quantitative differences in the amount of seromucoid found in each particular fraction. The total recovery of seromucoid was approximately 85 per cent for most individuals. These were picked at random from the seventy-five "normal" sera that have been fractionated. The remaining sixty sera gave results similar to those shown in Table VII.

The seromucoids subjected to the chromatographic technique as described in this paper are readily separated into five fractions. On rechromatography, where each of the original fractions is subjected to the entire fractionation procedure, seventy per cent or more of each fraction was recovered at the same sodium chloride concentration as the original fraction thus indicating that each fraction has distinctive elution characteristics under the experimental conditions (Table VIII).

Chemical determinations of the five fractions from three individuals are shown in Table IX. The fractions were prepared for chemical analysis by precipitation with phosphotungstic acid as used by Winzler for precipitating the whole seromucoid fraction from serum in his study of the

TABLE VIII

*Per Cent of Each Fraction Eluted by the Same Concentrations of NaCl upon Rechromatographing**

NaCl Molarity of Initial Eluent	Per Cent of Each Fraction Recovered in Each Eluent — NaCl Molarity of Refractionating Eluent				Total Per Cent Recovered
	0.02	0.08	0.135	0.35	
0.02 (first peak)	72	13	7	—	92
0.02 (second peak)	74	18	5	—	97
.08	—	72	18	—	90
0.135	—	7	78	6	91
0.35	—	—	18	69	87

* Values taken from reading at 280 mμ.

TABLE IX

*Chemical Ratios of the Five Fractions from Two Individuals**

Fraction	Individual	$\frac{\text{Tyrosine}}{\text{Protein}} \times 100$	$\frac{\text{Hexose}}{\text{Tyrosine}}$	$\frac{\text{Hexose}}{\text{Protein}} \times 100$	$\frac{\text{Hex NH}_2}{\text{Protein}} \times 100$	$\frac{\text{Hex NH}_2}{\text{Hexose}}$
0.02 (first fraction)	P.W.	3.9, 4.1	0.41, 0.44	2.4, 2.1	4.9, 5.2	2.6, 2.9
	G.W.	4.6, 4.8	0.73, 0.79	1.9, 1.8	7.4, 7.7	3.4, 3.8
0.02 (second fraction)	P.W.	5.8, 5.7	1.9, 2.1	14.2, 15.3	10.1, 10.6	0.81, 0.92
	G.W.	7.4, 6.9	2.3, 2.2	11.1, 12.1	11.2, 11.5	0.76, 0.81
0.08	P.W.	5.1, 4.6	2.3, 2.8	9.1, 9.9	7.9, 7.6	0.74, 0.82
	G.W.	5.2, 5.3	2.5, 2.9	10.3, 10.8	6.8, 6.2	0.62, 0.69
0.135	P.W.	8.9, 8.8	4.4, 4.7	40.1, 41.2	30.1, 32.4	0.82, 0.91
	G.W.	9.1, 9.2	4.7, 4.8	42.6, 43.7	29.5, 31.1	0.71, 0.78
0.35	P.W.	3.8, 3.6	5.1, 5.2	28.1, 27.4	16.2, 17.3	0.58, 0.51
	G.W.	4.6, 4.9	4.1, 3.8	21.4, 27.8	19.1, 20.8	0.42, 0.51

* Initial refers to individuals. Duplicate analyses were done on all samples.

chemistry of the seromucoid fraction (1). Hexose, tryosine, hexosamine, and protein were determined on all fractions. The various ratios were calculated as shown in Table IX.

The Analysis of the Carbohydrate Content of Each Fractionation by Paper Partition Chromatography

The determination of hexose and hexosamine shown in Table IX suggested that further investigation of the carbohydrate content of the various fractions might give additional data for showing chemical differences in the various fractions. Since orosomucoid has been shown to contain galac-

TABLE X

*Paper Partition Chromatography of the Serum Mucoid Fractions**

Fractions	Standards						Unknown Spots‡
	Sialic Acid	Fucose	Galactose	Mannose	Glucose NH_2	Galactose NH_2	
RF† standards							
E Py W	0.13	0.61	0.42	0.52	0.26	0.02	Trace .16 from gal. and gal. NH_2
B P W	0.13	0.56	0.27	0.40	0.26	0.03	
.02 (first peak)	+	+	0	+	+	±	
.02 (second peak)	+	+	±	+	+	+	.33, .71 E Py W
.08	±	+	±	+	+	±	
.135	+	+	+	+	+	+	.15, .72 B P W .73, .78 E Py W
.35	+	+	+	+	+	+	.78 E Py W .64, .79 B P W

* The following notations indicate the density of the spots: + = clear spot with an RF value the same as the carbohydrate standard; ± = weak spot with the RF value the same as the carbohydrate standard; 0 = no visible spot with an RF value the same as the carbohydrate standard.

† The RF designates the ratio obtained by dividing the centimeters from the origin to the standard by the distance in centimeters from the origin to the solvent front.

B P W = N-butanol; N-propanol; H_2O; 1:2:1; E Py W = Ethyl acetate; pyridine; H_2O; 10:4:3.

‡ The unknown spots are expressed as RF in the particular solvent system in which such spots were observed.

tose, mannose, fucose, hexosamine, and sialic acid (5), the assay for the various sugars were performed on all five fractions. Table X shows that the 0.35 fraction can be distinguished from the 0.135 fraction by the differences in the presence of unknown components. The second 0.02 fraction can be distinguished from the first 0.02 fraction by the presence of unknown components and the absence of galactose in the first 0.02 fraction. The 0.08 fraction can be distinguished from the first 0.02 fraction by showing a positive test for galactose. The 0.02 second fraction can be distinguished from the 0.08 fraction by the presence of unknown components with RF values of 0.33 and 0.71. The .08 fraction can be distinguished from the 0.135 and 0.35 fractions in lacking the unknown components that are in the latter two fractions.

DISCUSSION

A chromatographic method of fractionation using DEAE cellulose is reported which separates the seromucoid fraction of normal individuals into five fractions. This method is quantitative and reproducible. Seventy-five "normal" individuals have been studied and the sera of all have been found to contain the five fractions although there are quantitative differences found in the amount of seromucoid in each of the various fractions of different individuals. These fractions can be rechromatographed and show distinctive elution characteristics. Chemical analysis of the tyrosine, hexose, hexosamine, and protein content as well as assays for various carbohydrates by paper partition chromatography of the fractions also gave indications that the fractions were different from each other.

Many concentrations of sodium chloride were studied in evolving the elution process. The concentrations of sodium chloride finally used in the method described in this paper were those concentrations which produced sharp clear cut fractions with the relatively small amounts of sera employed. While the fractions described in this report are clearly reproducible in their chromatographic and chemical properties, they are not necessarily homogeneous. For example, using concentrations of sodium chloride between 0.135 M and 0.35 M it was possible to fractionate the 0.35 M fraction into three sub-fractions. Furthermore, the 0.135 M NaCl fraction could be sub-fractionated into at least three sub-fractions if this fraction was chromatographed on Cellex-P cation cellulose[5] using various sodium chloride concentrations. It is not clear at this time how these sub-fractions vary from individual to individual. The data does suggest however that the serum mucoid fraction is made up of a far larger number of molecular entities than was previously thought.

Using the method reported in this paper, differences in the seromucoid chromatographic patterns have been noted in individuals having various diseases. The clinical significance of these aberrations is not yet known.

SUMMARY

A reproducible and quantitative chromatographic method using DEAE cellulose has been developed for the separation of seromucoids into five fractions from human serum. The data strongly suggest on the basis of distinctive elution characteristics and chemical composition, that these five fractions represent different molecular entities. When this method was applied to the seromucoid fraction of seventy-five "normal" individuals, they all gave qualitatively the same pattern although there were quantitative differences observed in the various fractions of certain individuals.

[5] This cellulose was suggested by S. Harshman.

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FURTHER OBSERVATIONS ON AN INHERITED ANOMALY CHARACTERIZED BY PERSISTENCE OF FETAL HEMOGLOBIN[1]

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A recently recognized genetically determined anomaly is characterized by persistence of a high concentration of alkali-resistant hemoglobin in the erythrocytes in the absence of evidences of disease or other abnormalities (1, 2, 3, 4, 5, 6). Tentatively designated as "hereditary persistence of fetal hemoglobin" (3), the disorder has been encountered so far only in Negroes. Available information pertaining to this abnormality has been summarized in previous reports from this laboratory (5, 6). Additional data are provided by the present study, which is concerned with the nature of the alkali-resistant hemoglobin, the frequency of the anomaly, the mode of inheritance, and the interaction between this disorder and sickle-cell trait in persons who have inherited both abnormalities.

METHODS

Tests for the sickling phenomenon and determinations of red cell count, hematocrit value, hemoglobin concentration, percentage of reticulocytes, osmotic fragility of red cells, and serum haptoglobin were performed by methods employed in a previous study (5). Hemolysates were subjected to electrophoresis on filter paper using the method of Smith and Conley (7) and on agar gel with the method of Marder and Conley (8). Hemoglobin A_2 was measured after elution from starch block as described by Kunkel and his associates (9). Hemoglobin components were separated on chromatographic columns by the method of Allen and coworkers (10). Plasma iron turnover was determined by the technique of Bothwell and his associates (11).

The percentage of fetal hemoglobin in hemolysates was estimated by three methods:

1. The one-minute alkali-denaturation technique of Singer, Chernoff and Singer (12) was employed, using doubled volumes of reagents and hemolysates. This method was modified in the survey of large numbers of specimens in order to simplify the procedure. Red cells separated from blood collected in EDTA (disodium ethylenediaminetetraacetic acid) were washed once with 0.85 per cent sodium chloride solution and packed by centrifugation. The supernatant fluid was discarded and hemolysates prepared by addition of one volume of distilled water followed by freezing and thawing. The one minute denaturation test was performed with the following modifications: 20 cmm. of hemolysate, 1 ml. of N/12 potassium hydroxide and 2 ml. of precipitating solution were used. The test was performed at room temperature. The filtrate was inspected for the presence of undenatured hemoglobin and compared visually

[1] This investigation was supported in part by a research grant from the National Institutes of Health, U. S. Public Health Service.

with a preparation from a hemolysate known to contain 8 per cent fetal hemoglobin. This modified method regularly detected hemolysates containing 5 per cent or more fetal hemoglobin. Specimens with these elevated values were subjected to analysis by the quantitative technique.

2. The spectrophotometric method of Jonxis and Huisman (13) was used. Readings were made with a Beckman spectrophotometer at 544 mμ and the appropriate formula (14) employed to plot the rate of denaturation of the fetal hemoglobin. The specific reaction rate was calculated from these data as described by Shields and his associates (15).

3. A method of measuring hemoglobin components separated by electrophoresis on agar gel at pH 6.0 was developed. Hemolysate was pipetted into 3 to 4 cm. slits cut into the gel perpendicular to the direction of current flow. Following electrophoresis, rectangular blocks of agar of identical size, each containing a separate hemoglobin component, were cut from the agar and dissolved in 4 ml. of 12 N hydrochloric acid. A blank was prepared by dissolving in acid a similar rectangle of clear agar cut from the same plate. The optical densities of the acid solutions were determined using a spectrophotometer at 510 mμ, and the relative concentrations of the hemoglobin components were calculated. The nature of the soluble hemoglobin derivative was not investigated, but its spectral absorption curve differed from that of acid hematin prepared with N/10 hydrochloric acid. However, its absorption characteristics conformed to Beer's law over a wide range of concentrations. The method was compared with the starch block elution method of Kunkel and associates (9). Virtually identical values were obtained when mixtures of hemoglobins C and A were analyzed by the two methods. However, the agar method consistently gave a value for fetal hemoglobin that was 3 to 5 per cent higher than the value obtained by the alkali-denaturation method, when mixtures containing 60 to 80 per cent hemoglobin A and 40 to 20 per cent hemoglobin F were examined.

The increased mechanical fragility of sickled erythrocytes was utilized to separate sickled from discoid red cells. Erythrocytes containing hemoglobin S (from patients with sickle-cell anemia or from S-F heterozygotes) were subjected to various oxygen tensions. Two prepared gas mixtures containing (1) 93 per cent nitrogen and 7 per cent carbon dioxide, and (2) 20 per cent oxygen, 73 per cent nitrogen and 7 per cent carbon dioxide were introduced into a spirometer in appropriate amounts to provide the desired oxygen tension. Five ml. portions of blood containing EDTA were equilibrated with the gas in a silicone-coated syringe which contained 5 glass beads (diameter 5 mm.). The syringe was sealed and vigorously agitated on a mechanical shaker. The hematocrit value was determined before and after agitation. The percentage of fetal hemoglobin was measured in the cells resistant to trauma and was compared with the value for the untraumatized specimen.

The radioactivity of hemoglobin components which were labelled with Fe^{59} following the intravenous administration of Fe^{59} citrate was determined. Separation of hemoglobins S and F was accomplished by electrophoresis on agar gel. The hemolysate was pipetted into parallel slits cut in the agar and following its electrophoretic migration into the adjacent agar additional hemolysate was added repeatedly. A sufficiently large volume of hemolysate could be employed by this method so that the radioactivity of the hemoglobin fractions could be measured accurately. Following electrophoretic separation, agar containing each component was cut from the plate, partially dried to reduce the volume, and uniformly suspended in water in a volume of 6 ml. Radioactivity was determined in a well-type scintillation counter with a 3 inch crystal.

Sufficient counts were obtained to assure an accuracy of 95 per cent. The percentage of the radioactivity in the S and F fractions was compared with the percentage of the hemoglobin in these fractions.

RESULTS

Frequency of the Anomaly

Blood was obtained from 2200 consecutive Negro patients who had been referred to the routine Hematology Laboratory of the Outpatient Department of the Johns Hopkins Hospital. Fetal hemoglobin was estimated by the abbreviated alkali-denaturation technique. Accurate measurement of the amount of fetal hemoglobin was not made in this survey when the value was less than 5 per cent. Hemolysates estimated to contain 5 per cent or more were subjected to analysis by the precise alkali-denaturation method. In addition the fetal fraction was separated by electrophoresis on agar gel.

In the course of this survey 10 persons were found who had values for fetal hemoglobin in excess of 5 per cent. Of these, two were patients with sickle-cell anemia (8% and 12%), and one patient had sickle-cell-thalassemia disease (10% hemoglobin F). Two patients with elevated values had aplastic anemia[2]. Three other patients were observed to have moderately high values for fetal hemoglobin (6.9, 8.3 and 11.0%) in the absence of an obvious cause. None of these had other hemoglobin abnormalities. Family studies were not performed and the reason for the increased fetal fraction was not determined. *Two patients had extremely high values for fetal hemoglobin (25 and 28%).* The first of these was under treatment for bronchiectasis but had no other evidence of a blood disorder. A study of members of his family revealed similarly high values in persons of each of three generations. The patient with 28 per cent fetal hemoglobin had sarcoid with pulmonary involvement, with no apparent reason for the high fetal hemoglobin value. Family members were not available for study.

Genetic Observations

Five families were encountered in which high concentrations of fetal hemoglobin occurred in the absence of anemia or apparent illness. All

[2] Both of these were young adult males whose aplastic anemia was of undetermined cause. In one, fetal hemoglobin ranged between 7 and 10 per cent on repeated determinations during a period of 7 months of observation. The second patient had an initial value of 8 per cent with a subsequent rise to 20 per cent over a period of three weeks. During the ensuing six months values between 16 and 20 per cent were repeatedly obtained. Electrophoretic examination of the hemoglobin showed no abnormality other than the presence of fetal hemoglobin in increased amount in these cases.

of these were discovered in the course of surveys to determine the frequency of hemoglobin abnormalities in the Baltimore Negro population. The anomaly was traced through three generations in each kindred. In two of these families hemoglobin S was also present; both of these kindreds have been described in previous reports (5, 6). Figure 1 shows the hemoglobin phenotypes of the members of these families.

Twenty-seven persons with hemoglobins A and F were identified. There were 14 males and 13 females. Alkali-resistant hemoglobin ranged between 18 and 33 per cent with a mean of 26 per cent (Fig. 2). In each instance studied a person with an increased fetal fraction had one parent with this abnormality. The children of an A-F father and an A-S mother showed four hemoglobin phenotypes (A-A, A-S, A-F and S-F). Five persons had hemoglobins S and F and in these hemoglobin A could not be detected. In this S-F group alkali-resistant hemoglobin ranged between 27 and 33 per cent with a mean of 29 per cent. In each instance hemoglobin S had been acquired from one parent, hemoglobin F from the other. Each child of an S-F heterozygote obtained one but not both of the abnormal hemoglobins.

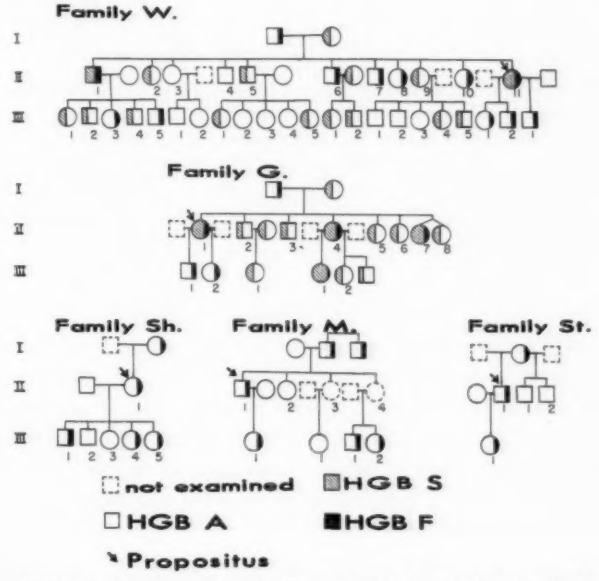

FIG. 1. Hemoglobin phenotypes in 5 Negro families in which hereditary persistence of fetal hemoglobin was discovered. The diagram indicates the proportions of hemoglobins as they were measured. In family G the child (III-1) of the S-F heterozygote (II-4) has typical sickle cell anemia, and presumably derived an S gene from the father, who was not available for examination.

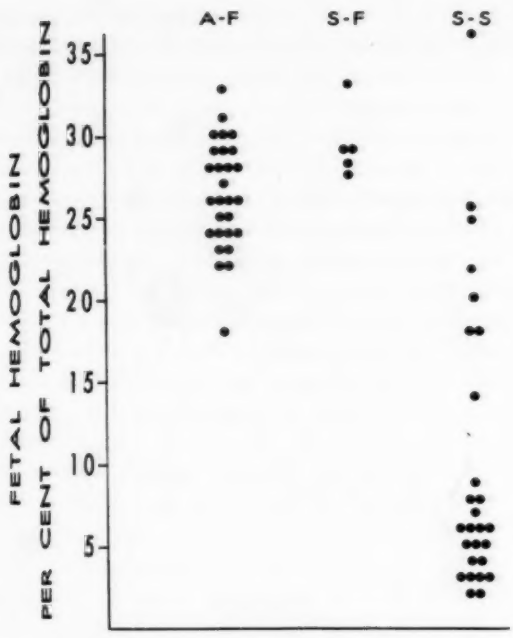

FIG. 2. Percentage of fetal hemoglobin, as measured by the alkali-denaturation technique, in hemolysates of persons with hereditary persistence of fetal hemoglobin and of patients with sickle-cell anemia. Persons heterozygous both for the fetal anomaly and for hemoglobin S (S-F) have a mean value for fetal hemoglobin which is higher than that of the A-F heterozygotes. This difference may be significant and suggests that hemoglobin A is more effective in suppressing synthesis of fetal hemoglobin than hemoglobin S.

Characteristics of the Fetal Hemoglobin

The "fetal" hemoglobin of this inherited anomaly has been so designated because of its resistance to denaturation by alkali. Additional studies were performed to characterize further this hemoglobin and to investigate its relationship to the fetal hemoglobin of umbilical cord blood and to that which is encountered in the blood of patients with sickle-cell anemia.

Electrophoretic Properties

Hemolysates of all of the members of the five families were subjected to electrophoresis on filter paper at pH 8.6 and on agar gel at pH 6.0. During filter paper electrophoresis the fetal fraction migrated to a position between hemoglobins A and S, but was not clearly separated from either. The

electrophoretic pattern of individuals with hemoglobins A and F resembled the normal pattern, although there was a definite trial in the position of fetal hemoglobin. The S-F heterozygotes could not be distinguished from persons with sickle-cell anemia by this technique. The rate of migration of the fetal fraction was identical to that of the fetal hemo globin of umbilical cord blood and to that of sickle-cell anemia.

Electrophoresis on agar gel provided clear separation of the fetal fraction, which migrated more rapidly toward the cathode than did hemoglobins A and S (Fig. 3). The characteristics of the fetal fraction were similar to those of the fetal hemoglobin of umbilical cord blood. Rate of migration of the fetal component was not significantly related to the concentration of hemoglobin in the hemolysate, in contrast to the behavior of hemoglobins A, S, C and D. Thus the fetal component maintained a rounded

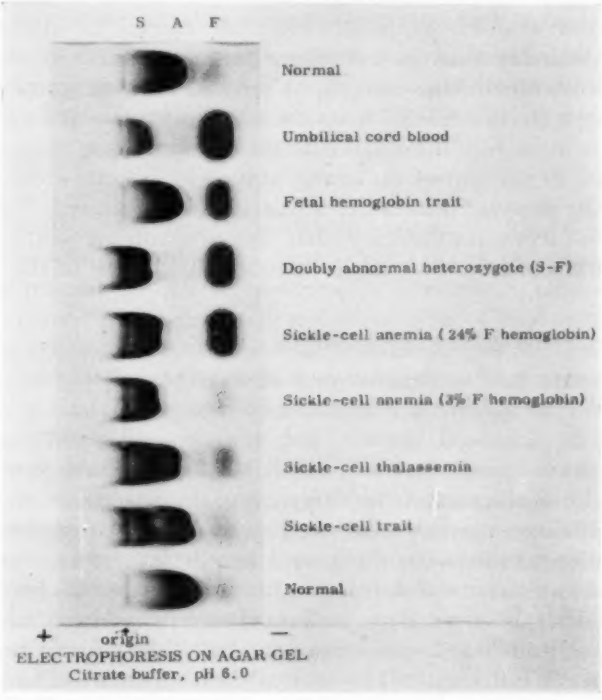

FIG. 3. Hemolysates from various sources are compared after electrophoresis on agar gel. Note the wide separation, the rounded form and the uniform migration of the fetal hemoglobin spots. The pattern of the S-F heterozygote cannot be distinguished from that of the patient with sickle-cell anemia whose hemolysate contained a high concentration of fetal hemoglobin.

TABLE I

Specific Rates of Denaturation of Fetal Hemoglobin by Alkali

Group	Number of Individuals	Number of Determinations	Specific Reaction Rate	
			Range	Mean*
A-F	8	9	0.072–0.154	0.128
S-F	2	9	0.076–0.113	0.097
Cord blood	9	9	0.060–0.109	0.079
S-S	1	4	0.066–0.079	0.072

* The average value for each individual who had multiple determinations was obtained. The mean value for the group was determined from these individual averages.

appearance during electrophoresis and did not acquire the bullet-shaped or swept-back appearance of the other hemoglobins.

Chromatographic and Spectrophotometric Characteristics[3]

Hemoglobin fractions from the hemolysate of an S-F heterozygote were separated by column chromatography using the ion exchange resin IRC-50. The first fraction eluted from the column contained 30 per cent of the total hemoglobin; the alkali-resistant fraction of the same hemolysate constituted 29 per cent of the hemoglobin. The fraction eluted from the column was analyzed with a recording spectrophotometer. The spectral absorption curve was identical to that obtained with the fetal hemoglobin of the umbilical cord, showing the characteristic notch in the ultraviolet spectrum.

Resistance to Denaturation by Alkali

The specific rate of denaturation of fetal hemoglobin by alkali was studied in four groups: the A-F and S-F individuals in our families, a patient with sickle-cell anemia, and newborn infants. The results in each group are summarized in Table I. More data would be necessary to establish the significance of the differences in the mean value of the various groups. However, the values for the two S-F persons suggest that the apparent differences between the groups may be no greater than the differences among members of a single group. In the case of one S-F heterozygote, 7 determinations were performed, specific reaction rates ranging between 0.076 and 0.097 with a mean of 0.085. The other S-F heterozygote gave values of 0.101 and 0.113 on two determinations. The overlap of values in all groups suggests a similarity of the reaction kinetics of the alkali-resistant hemoglobin of these groups.

[3] Dr. Harvey Itano and Miss Elizabeth Robinson, Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland, provided valuable assistance in this study.

Clinical and Hematological Data

None of the persons with hemoglobins A-F displayed clinical manifestations or hematological abnormalities attributable to the inherited anomaly. Anemia was not present and careful examination of the red cells on stained blood smears showed no consistent deviation from normal. The percentage of hemoglobin A_2 was in the normal range. The mean corpuscular volume, mean corpuscular hemoglobin concentration and osmotic fragility of the red cells were normal. Reticulocytes were not increased, the icterus index was not elevated and the serum haptoglobin was normal.

The five persons with hemoglobins S-F had approximately 70 per cent sickle hemoglobin in their red cell hemolysates, but there were no symptoms of sickle-cell disease and the body habitus was normal. Examination of the conjunctival blood vessels with the corneal microscope did not show the characteristic abnormalities of blood flow seen in these vessels in patients with sickle-cell anemia[4] (16). Sickling of the red cells was readily induced with sodium metabisulfite, but anemia, reticulocytosis and hyperbilirubinemia were not encountered. Serum haptoglobin was normal. The turnover of plasma iron, measured in J. G. (II-1 of Family G), was 0.55 mg. iron per day per 100 ml. blood, a normal value.

Benign Nature of Heterozygosity for Hemoglobins S and F

The S-F heterozygotes remain well while patients with homozygous sickle-cell disease are anemic even though their red cell hemolysates may contain high percentages of fetal hemoglobin. The benign nature of the heterozygous disorder has been attributed to the effect of fetal hemoglobin in preventing the occurrence of the sickling phenomenon at physiological oxygen tensions (5). An explanation must be provided for the apparent failure of similarly high levels of fetal hemoglobin to protect the patient with sickle-cell anemia.

First it was necessary to establish that symptomatic sickle-cell anemia can in fact be associated with levels of fetal hemoglobin as high as those of the S-F heterozygotes. Accordingly, blood was obtained from 107 patients with homozygous sickle-cell disease from the Hematology Clinic. Electrophoresis of hemoglobin on agar gel was employed as a screening technique. The alkali-denaturation method was used in 27 cases, including all of those with values for fetal hemoglobin estimated to be as high as 15 per cent. The results of this study are shown in Figure 2. Of the 107 patients only 7 had amounts of fetal hemoglobin in excess of 15 per cent and only two had values as high as those encountered in the S-F heterozygotes. Although the majority of the patients in this survey were adults, values

[4] Examination of the conjunctival blood vessels was performed by Dr. David Paton as part of an extensive study of the conjunctival vessels in sickling disorders.

TABLE II

Patients with Sickle-Cell Anemia, of 107 Examined, with Values for Fetal Hemoglobin in Excess of 15 Per Cent

Patient	Age	Fetal Hemoglobin Per Cent
J. G.	9 mos.	36
D. P.	22 mos.	25
R. W.	2 years	26
L. W.	4 years	17
J. W.	5 years	21
G. J.	7 years	21
U. F.	30 years	18

for fetal hemoglobin in excess of 15 per cent were encountered in children, with one exception (Table II). All of the patients were anemic. One case is of unusual interest for the patient was found to have 36 per cent fetal hemoglobin, a value higher than that of any of the S-F heterozygotes. Despite this large proportion of fetal hemoglobin, the patient had extremely severe sickle-cell anemia.

The patient was a nine-month-old infant who had displayed lethargy and fever for six days. On examination she appeared to be profoundly anemic. The hematocrit value was 18 per cent and hemoglobin concentration 3 grams per 100 ml. Electrophoresis of hemoglobin showed only S and F components. The alkali-resistant fraction constituted 36 per cent of the total. Symptoms subsided following transfusion. Three months later there was recurrence of anemia (hemoglobin 3.8 grams per 100 ml.). Splenomegaly was noted for the first time. Convulsions were followed by death before transfusions could be administered.

In this instance fatal sickle-cell anemia was associated with an extremely high level of fetal hemoglobin. The electrophoretic pattern of the hemolysate of the patient was indistinguishable from that of the apparently healthy and non-anemic S-F heterozygote. It is evident that in this infant the proportions of sickle and fetal hemoglobin were not uniform throughout the red cell population. Fetal hemoglobin decreases progressively during the first year of life, so that most recently formed cells contain the lowest percentage of fetal hemoglobin. This patient's disease was undoubtedly due to the intravascular sickling of younger cells which contained the highest proportions of sickle hemoglobin. The striking differences in clinical manifestations of the two disorders in older persons similarly could be explained by a difference in distribution of fetal hemoglobin in the red cell populations. Therefore, experimental studies were performed to investigate the manner of distribution of hemoglobin F in the red cells of an S-F heterozygote and of a patient with sickle-cell anemia.

Distribution of Fetal Hemoglobin in the Red Cell Population in the S-F Heterozygote and in Patients with Sickle-Cell Anemia

Experiments Based on Selective Destruction of Sickled Erythrocytes

Blood was obtained from an S-F heterozygote and from patients with sickle-cell anemia who had been demonstrated to have increased amounts of fetal hemoglobin. The available patients with homozygous sickle-cell disease did not have fetal fractions as large as those of the S-F heterozygotes. Fresh blood containing EDTA was equilibrated with mixtures of oxygen, nitrogen and carbon dioxide so that various oxygen tensions were obtained. The blood specimens were subjected to vigorous agitation with glass beads, a procedure which preferentially destroys sickled erythrocytes. The fetal fraction was measured in aliquots of the original specimens and in hemolysates of the cells which were resistant to mechanical trauma. The results of a group of these experiments are shown in Table III. Trauma caused much more rapid decrease in the hematocrit value when the blood was deoxygenated than when fully oxygenated, demonstrating the enhanced susceptibility of sickled erythrocytes to destruction by mechanical injury. This effect was as great in the blood of the S-F heterozygote as in the blood from patients with sickle-cell anemia. As compared with the initial specimen, there was only a slightly higher value for the percentage of fetal hemoglobin in the most resistant cells of the S-F heterozygote. This experiment provides no evidence that the relatively resistant

TABLE III

Fetal Hemoglobin Content of Red Cells Resistant to Mechanical Trauma

Source of Red Cells	Experiment	Oxygen Concentration	Hematocrit Value		Fetal Hemoglobin		
			Before Agitation	After Agitation	Before Agitation	After Agitation	Per Cent Change
		%	%	%	%	%	
S-F heterozygote	1	20.0	41.0	32.0	28.8	28.7	−0.3
		0.5	41.0	21.0	28.8	29.8	+3.4
	2	0.5	41.0	21.0	29.1	29.7	+2.1
	3	0.5	41.0	15.5	29.1	29.8	+2.4
	4	0.5	39.5	21.5	29.6	30.1	+1.7
Sickle-cell anemia	1	20.0	30.0	25.0	17.8	18.7	+5.6
		0.5	30.0	14.0	17.8	19.4	+9.0
	2	0.5	28.0	16.0	17.6	20.2	+14.5
	3	0.5	29.0	16.0	22.6	24.8	+9.7

cells of the S-F heterozygote contained appreciably higher levels of fetal hemoglobin than the more susceptible cells. It appears, therefore, that the distribution of hemoglobin F in the red cells of the S-F heterozygote is quite homogeneous.

This study is not so satisfactory to demonstrate the distribution of hemoglobin F in the red cells of patients with sickle-cell anemia. The initial concentration of hemoglobin F in these patients was relatively low so that direct comparisons with the S-F data cannot be made. Furthermore, in sickle-cell anemia the life span of the red cells is short, and cells containing the highest concentrations of hemoglobin S are presumably most rapidly destroyed *in vivo*. Therefore, venous blood does not contain a representative sample of red cells as they are produced in the bone marrow, but rather the residua after sequestration of the most susceptible cells has occurred. To circumvent these difficulties, other experiments were performed.

Experiments Utilizing Radioactive Iron to Measure the Relative Size of Circulating Pools of Hemoglobins S and F

The distribution of radioactive iron between the sickle and fetal fractions of hemoglobin was observed following administration of radioiron to an S-F heterozygote and to a patient with sickle-cell anemia. The latter was a child of 22 months whose parents were demonstrated to have sickle-cell trait. The patient had hematocrit values ranging between 24 and 27 per cent; there were 15 per cent reticulocytes, and the fetal hemoglobin fraction separated on agar gel was approximately 30 per cent. Fe^{59} citrate was administered intravenously to both subjects. Hemolysates were prepared from blood samples obtained at intervals thereafter, and the S and F components separated by electrophoresis on agar gel. The percentage of the hemoglobin and of the radioactivity in the two components was determined.

The results obtained in the two subjects were strikingly different (Table IV). The hemolysates of the S-F heterozygote contained about 35 per cent fetal hemoglobin, and about 35 per cent of the radioactivity was in the fetal fraction. Thus, the radioactive iron per gram of hemoglobin was the same in the two fractions. Determinations made on the 8th and 54th days after administration of radioactive iron showed no significant difference.

The patient with sickle-cell anemia displayed clear evidences of a hemolytic anemia. Therefore, the initial measurements were made on the third day after administration of radioactive iron, presumably before many of the newly formed and labelled erythrocytes were destroyed. At this time the specific activity of the fetal fraction was much less than that of the sickle fraction. This result can be explained by differing degrees of

TABLE IV

Hemoglobin Fractions Separated on Agar Gel after Administration of Radioactive Iron

	S-F Heterozygote		Sickle-cell Anemia	
	Day 8	Day 54	Day 3	Day 7
Hemoglobin in fetal fraction (Per cent of total hemoglobin)	33.5	35.0	31.5	30.0
Radioactivity of fetal fraction (Per cent of total radioactivity)	35.2	36.4	14.9	20.3

dilution of the tagged hemoglobin by the circulating unlabelled hemoglobin. Presumably hemoglobins S and F were distributed heterogeneously within the red cells, and those cells containing the highest proportions of hemoglobin S had been preferentially destroyed *in vivo*. Therefore, the circulating pool of hemoglobin S available to dilute the radioactive hemoglobin was relatively smaller than the pool of hemoglobin F (in relation to the proportions of these hemoglobins in newly formed red cells). By the seventh day after administration of radioactive iron, the radioactivity of the fetal fraction showed a relative increase, an observation which could be accounted for by preferential destruction of the labelled cells containing the largest amounts of hemoglobin S.

The results of the radioactive iron study in the S-F heterozygote do not give direct evidence that the distribution of hemoglobins S and F is uniform, since a hemolytic disorder was not present and all of the red cells apparently had a normal life span. However, the data demonstrate the accuracy of the method and also suggest that the results in the patient with sickle-cell anemia cannot be accounted for by a different rate of appearance of the labelled iron in the two hemoglobin components. Ranney and Kono (17) observed in other genetic abnormalities of hemoglobin formation that radioactive iron appears at an equal rate in the two major hemoglobin components.

DISCUSSION

In this benign inherited hemoglobinopathy the only abnormality which has been recognized is the occurrence of alkali-resistant hemoglobin in high concentration. Observations described in the present study add to evidence previously obtained (5) that this peculiar hemoglobin is identical to the fetal hemoglobin of the newborn child. It cannot be distinguished from fetal hemoglobin by electrophoresis in various buffers or by elution from chromatographic columns. It displays the unique behavior of fetal hemoglobin during electrophoresis on agar gel at pH 6.0. The kinetics

of its denaturation by alkali are similar to those of fetal hemoglobin. Furthermore, it has the same distinctive absorption curve in the ultraviolet spectrum. Proof of identity will require complete analysis of the amino acid sequences of the globin moiety, but present evidence justifies the reasonable presumption that the alkali-resistant hemoglobin of the inherited anomaly is indeed fetal hemoglobin.

The survey of a Negro population in our clinic revealed that one person in 2200 examined definitely had the disorder, as proved by family studies, and one other is presumed to have it although family studies were not performed. In a survey of 1000 Negroes in Philadelphia, one person with a similarly high value for fetal hemoglobin was encountered (18). It appears, then, that the frequency of the anomaly is about one in 1000 Negroes. Its occurrence in other racial groups has not been described.

The pattern of inheritance is clearly shown by the pedigrees described in this and in preceding reports. The anomaly appears to be transmitted by an autosomal mutant gene allelic with the gene determining the abnormality of hemoglobin S. In each of the families in which persons were heterozygous both for the fetal anomaly and for hemoglobin S, hemoglobin F was acquired from one parent and hemoglobin S from the other. Hemoglobin A could not be detected in hemolysates from these doubly abnormal heterozygotes, even when a sensitive method employing agar gel was employed (5). Each child of an S-F heterozygote obtained one but not both of the abnormal hemoglobins. Hemoglobin C has also been encountered with the fetal anomaly; the C-F heterozygote similarly was found to have no hemoglobin A (19). McCormick and Humphreys (20), studying an anemic patient thought to represent a C-F heterozygote, concluded from indirect evidence that hemoglobin A was present; but its existence was not actually demonstrated nor was the possibility of C-thalassemia disease excluded as the basis of the patient's disorder.

We have previously stated our hypothesis for the genetic basis of the fetal hemoglobin anomaly (6). The mutant gene in persons with hereditary persistence of fetal hemoglobin appears to be at the locus which determines the structure of the β chain of the globin. The effect of the mutation is probably to prevent formation of functional β chains so that hemoglobin A is not produced. As a secondary phenomenon there is compensatory production of fetal hemoglobin, synthesis of which is normally suppressed. The γ chain, peculiar to the globin structure of fetal hemoglobin, is determined at a genetic locus independent of the β chain locus, so that hemoglobins S and F in these families cannot be directly controlled by allelic genes. Persons having the fetal anomaly show no evidence of interference with the total production of hemoglobin, since anemia is not present and the red cells are not hypochromic. In this respect

hereditary persistence of fetal hemoglobin is strikingly different from thalassemia, in which there is not adequate compensation for the retarded production of hemoglobin A. The differences between the fetal anomaly and thalassemia minor previously have been emphasized (5). The interrelationship between these disorders is currently under study in our laboratory in a large family in which both abnormalities are present.

In the five families described in the present study 32 persons had elevated concentrations of fetal hemoglobin, with values ranging from 18 to 33 per cent. Five persons also had hemoglobin S in their red cell hemolysates. In this latter group the non-fetal fraction consisted of hemoglobin S, which constituted from 67 to 73 per cent of the total hemoglobin. Despite the very large proportions of sickle hemoglobin, these persons were well and displayed no evidence of anemia. The absence of jaundice, and the normal reticulocyte count, serum haptoglobin and plasma iron turnover indicate that there was not a compensated hemolytic process. In contrast, persons with homozygous sickle-cell disease displayed pronounced or even fatal anemia even though the percentage of fetal hemoglobin in their red cell hemolysates was as high as in the nonanemic S-F heterozygotes. The remarkable clinical differences between these disorders in which the hemolysates are indistinguishable are accounted for by our studies. There is good evidence that sickle hemoglobin causes shortening of the life span of the red cells only if the sickling phenomenon occurs *in vivo*. Our experiments indicate that in the S-F heterozygotes fetal hemoglobin is uniformly distributed throughout the red cell population. Presumably every red cell has sufficient fetal hemoglobin to prevent its sickling at physiological oxygen tensions. Accordingly, all of the cells have a normal life span. In sickle-cell anemia, on the other hand, the distribution of fetal hemoglobin is heterogeneous, an observation reported by Singer and Fisher (21) and confirmed by the present studies. Those cells with the highest proportions of sickle hemoglobin are most susceptible to sickling *in vivo* and therefore to rapid destruction. Cells containing relatively high concentrations of fetal hemoglobin tend to resist destruction. As a result of the selective removal of cells containing lesser amounts of fetal hemoglobin, the circulating blood contains a mean percentage of fetal hemoglobin higher than that of newly formed red cells. The lack of correlation in sickle-cell anemia between severity of anemia and concentration of fetal hemoglobin in the circulating blood is readily explained by these observations.

SUMMARY

A benign inherited anomaly of hemoglobin synthesis, manifested by persistence of high concentrations of alkali-resistant hemoglobin, has been

studied in 5 families. The abnormal hemoglobin of the inherited disorder appears to be identical with the fetal hemoglobin of the newborn child. Genetic investigations suggest that the abnormality is the result of a mutation at the locus at which the structure of the β chain of the globin moiety of hemoglobin is determined. The primary effect of the mutant gene probably is to prevent formation of functional β chains so that hemoglobin A is not produced. As a secondary and compensatory result, fetal hemoglobin is synthesized in quantities sufficient to prevent the occurrence of anemia or of hypochromia of the erythrocytes.

Persons heterozygous both for the fetal anomaly and for hemoglobin S have approximately 70 per cent hemoglobin S and 30 per cent hemoglobin F in their red cell hemolysates, yet have no evidence of a hemolytic disorder. In contrast, patients with homozygous sickle-cell disease may have severe anemia at times when their red cell hemolysates contain these same proportions of hemoglobins S and F. The remarkably different clinical manifestations are explained by differences in distribution of the fetal hemoglobin among the red cells in the two disorders. In the S-F heterozygotes the uniform distribution of hemoglobin F among the red cells apparently prevents sickling of any of the cells at physiological oxygen tensions. In homozygous sickle cell disease fetal hemoglobin is heterogeneously distributed so that many cells contain insufficient hemoglobin F to protect them from sickling *in vivo* and are therefore rapidly destroyed.

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ELECTRON MICROSCOPY OF THE CEREBRAL CORTEX[1]

I. The Ultrastructure and Histochemistry of Synaptic Junctions[2]

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Examination of the cerebral cortex with the light microscope has revealed a paucity of synaptic junctions although electrophysiological studies suggest numerous types of endings. Particularly conspicuous is the lack of boutons terminaux. The difficulty in demonstrating synapses in the cortex with routine staining methods is attributable to two factors: (a) the limited resolving power of the light microscope, which does not ordinarily permit visualization of terminal cell processes smaller than 0.2 micron and (b) the capricious nature and limited reliability of silver staining methods. As a consequence, relatively little is known concerning the fine structural organization of cortical synapses.

Numerous regions of the mammalian nervous system have been examined with the increased resolving power of the electron microscope, e.g., sensory receptors (1, 2, 3), the neuromuscular junction (4), and certain synaptic junctions (5 to 8). From such studies, it is now possible to identify the various components of the nervous system, i.e., nerve cells and their processes (9, 10), the various neuroglial cell types (9, 10), and a variety of synaptic relationships (5 to 8). Since the criteria for the identification of the various elements have been treated adequately in the literature they will be given only brief treatment in this report.

However, the structural organization of cortical synapses remains obscure. The aim of this report is to describe two types of synapses in the cerebral cortex examined with the electron microscope: (a) axo-dendritic endings and (b) axo-somatic endings. Particular emphasis is directed to their ultra-structure and to histochemical localization of cholinesterase.

MATERIALS AND METHODS

Small pieces of visual and auditory cortex were removed from surgically prepared monkeys, cats and rats. They were fixed in buffered osmium tetroxide or potassium

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permanganate for 1 hour at 4°C. The tissues were dehydrated in acetone or ethanol and embedded in araldite or Shell Epon resin #812 according to standard techniques. Thick sections (0.1 to 0.2 microns) were cut on a Porter-Blum (Servall) microtome and studied with the phase contrast microscope. Contiguous thin sections (200 to 300 Ångstroms) were examined in an RCA EMU 3-c electron microscope and an Elmiskop I. Additional tissues were prepared and stained for cholinesterase with the techniques described by Lehrer and Ornstein (11) and Barrnett and Palade (12). Only minor changes in these procedures were necessary for staining the central nervous system.

OBSERVATIONS

General Ultrastructure of the Cortex

Examination of the mammalian cerebral cortex with the electron microscope reveals a complex network of nerve cells, neuroglia and their numerous processes, in intimate contiguity. Figures 1 and 2 are low magnification electron micrographs showing the appearance of typical sections through the cortex. It becomes immediately apparent that cells and their processes occupy most of the space available and any extracellular space is quite small. Numerous studies have consistently demonstrated extremely small extracellular spaces in the central nervous system (10, 13). Since various fixatives and embedding techniques have been employed it seems unlikely that this is a significant artifact of preparation. The obvious discrepancy between inulin and chloride spaces and those observed in electron microscopy have been discussed elsewhere (10, 13, 16). In Figure 1 a small capillary (C) is seen with an erythrocyte in its lumen. Note that neuroglial pale processes (G) surround most of the capillary endothelium and thus a glia sheath separates most of the vascular bed from the nerve cells. A more complete description of capillaries in the central nervous system will be the subject of another report. In Figure 2 a small myelinated fiber (M) and numerous other axons and dendrites (D) are seen closely applied to one another. In those cases in which the section passes through a region containing large neurons (Fig. 2), the close spatial relationships are also evident. In Figure 2 the nerve cell nucleus (N) is bounded by the typical double nuclear membrane and demonstrates nuclear pores. A large dendrite (D) is seen in close contiguity with the plasma membrane of the neuron (see arrows) and myelinated nerve fibers (M) are also encountered. At this relatively low order of resolution, (Figs. 1 and 2), definitive synaptic junctions are not apparent. It must be emphasized that no attempt was made to accurately localize the various layers of the cortex in this study. Apical and basal dendrites and cellular layers were identified in thick sections examined with a phase microscope and then contiguous thin sections were examined with the electron microscope.

Ultrastructure of Synapses in the Cortex

During the past few years numerous studies have appeared describing the fine structure of synaptic junctions in various regions of the nervous system (5 to 8). These accounts have shown a remarkable uniformity in structure. Most synapses are characterized by closely applied pre- and postsynaptic processes, separated by a synaptic cleft approximately 200Å wide. Presynaptic processes usually contain accumulations of mitochondria and "synaptic vesicles", whereas the postsynaptic processes are unremarkable in fine structure. Localized thickenings are seen in one or both of the synaptic membranes. For a more comprehensive review of this subject see de Lorenzo (6, 8). Early examination of the mammalian cerebral cortex revealed numerous synaptic junctions identifiable by the above criteria. In fact, two types of synapses were seen and classified on the basis of their fine structure, i.e., axo-dendritic and axo-somatic (13, 14). Figure 3 is an electron micrograph which demonstrates several of the axo-dendritic variety. In the figure, two large dendrites (D) are seen, which have been cut longitudinally and are characterized by endoplasmic reticulum. In addition numerous other dendrites are seen which have been cut in cross section. It is of interest that only the membraneous component of the endoplasmic reticulum is seen at the extreme terminals of the dendrites and in dendritic spines. This is best seen in the longitudinal sections through the dendrites.

At the regions designated by the arrows, axo-dendritic synapses are evident. Note that the presynaptic axons (A), contain "synaptic vesicles" but that in these small endings, mitochondria are not conspicuous. The postsynaptic processes (dendritic spines D) contain only membranes derived from the endoplasmic reticulum. Localized thickened regions of the synaptic membranes are readily apparent and the "synaptic vesicles" are usually clustered at these regions (5, 6). Particularly conspicuous is the fact that the postsynaptic membrane is more dense than the presynaptic. This is seen in Figures 3 and 4. In areas where axons terminate upon the main trunk of the dendrites, both the dendrites (D) and the terminal axons contain mitochondria. Vesicles (V) abound in the presynaptic process (Fig. 4). Gray has shown that about 47 per cent of dendrites at synaptic junctions are devoid of mitochondria and that an additional 43 per cent of synaptic dendrites contain only one mitochondrion (14). Our observations are in agreement and suggest that only the less numerous but large axo-dendritic synapses as seen in Figure 4 contain accumulations of mitochondria. The more numerous endings on dendritic spines usually lack mitochondrial profiles. The arrows in Figure 3 and 4 designate the localized thickening of the synaptic membranes typical of axo-dendritic junc-

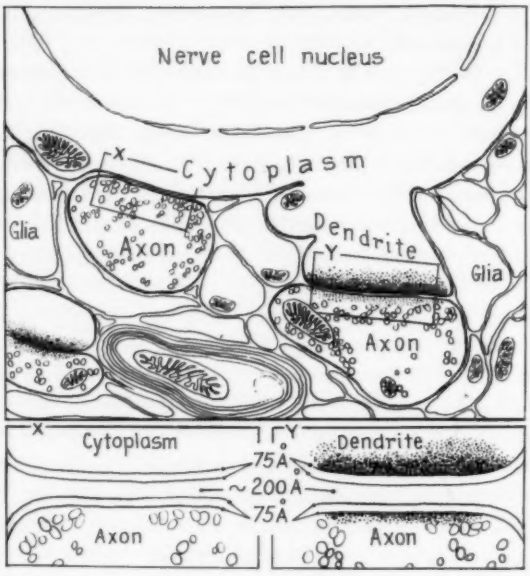
tions. Small myelinated fibers (M), frequently less than 0.1 micron in diameter are encountered.

It is apparent in Figures 3, 4 and 5 that many axon terminals (identifiable as such by their "synaptic vesicles") are in close contiguity with dendritic processes, yet show no particular membrane specialization. The question quite naturally arises whether the thickened membrane is out of the plane of section. One cannot be certain that all axo-dendritic synapses must be associated with membrane thickening. However, serial sectioning of such regions allows observation of sections some 300Å apart. Several terminal axons have been studied in this manner and fine structure specialization of the appositional membranes was not observed. Thus either the thickened regions escaped detection, which seems unlikely, or a further subclassification of axo-dendritic synapses should be made. The evidence in this study supports the latter interpretation (see below).

It is of particular interest that both Gray's (14) and our earlier studies (13) have shown that axo-somatic contacts are also present, but are so consistently different in certain fine structural details, that a useful second type classification has been made. As in the above type, the presynaptic processes sometimes contain mitochondria and invariably contain "synaptic vesicles". However, the thickened regions of the synaptic membranes is conscipuously absent in our preparations. Gray's observations are in essential agreement with ours but his tissues were routinely stained with PTA. His observations show occasional but small areas of membrane density in axo-somatic synapses which may very well be the result of the staining procedure employed. Our control preparations are unstained and show no conspicuous membrane specializations. Recent observations on the ultrastructure of synaptic membranes (Figs. 6 and 8) are totally in agreement with Gray's (14). The pre- and postsynaptic membranes consist of unit membranes as first described by Robertson in other tissues (15). The synaptic cleft is approximately 200Å wide (Figs. 6 and 8). These general observations on the ultrastructural relationships of cortical synapses are schematically represented in Text Figure 1.

Acetyl-cholinesterase Staining

The fine structure of synapses in the cortex suggests at least two kinds of endings and electrophysiological studies have shown functional differences in axo-dendritic synapses as contrasted with axo-somatic endings (19). It, therefore, seemed advisable to stain the cortex for acetyl-cholinesterase in an attempt to further elucidate any relation of fine structure and function that might exist. The methods described by Lehrer and Ornstein (11) and Barrnett and Palade (12) were applied to small pieces of



TEXT FIG. 1. This is a schematic representation of the ultrastructure of synapses in the cortex. Two kinds of synapses are evident, i.e., axo-dendritic and axo-somatic. Axo-dendritic synapses are characterized by presynaptic axons which contain "synaptic vesicles" and occasional mitochondria, in close contiguity with dendritic processes. The synaptic cleft measures about 200Å. The pre-and postsynaptic appositional membranes demonstrate a characteristic thickening along most of the region of the contiguity. Axo-somatic synapses likewise demonstrate close contiguity of appositional membranes and presynaptic vesicles. However, the synaptic membranes do not appear to demonstrate any fine structural specialization which is characteristic of the other type of ending.

Insert X shows the ultrastructure of the synaptic membranes in an axo-somatic synapse and insert Y the fine structure of the axo-dendritic variety. For details, the reader is referred to the text.

cortex. The latter method proved more successful in our hands after some adjustments and experience. The results of cholinesterase staining of cortical synapses are shown in Figures 4 through 8. In Figure 4, two synaptic regions characterized as axo-dendritic and identified by the X labeled arrows show a heavy, dense deposit in the region of the synaptic membranes. Likewise, the vesicles in the presynaptic axons appear much denser than in an unstained control section such as Figure 3. Note, however, that in the large axo-dendritic synapse in the middle of the figure the dendritic (D) and axonic appositional membranes (unlabeled arrows) do not appear to be significantly denser than the controls (Fig. 3). Likewise the vesicles in this synapse are unstained. Curiously, the mitochondria seem to have

been stained, if this can be inferred from their extremely dense appearance (Figs. 4 and 5). Figure 5 is a section which was stained for cholinesterase in a region with many axo-dendritic synapses. At each of the regions designated by the arrows an extensive dense area is seen in the synaptic junction which all but obliterates the appositional membranes. The ultrastructure of such an area is shown in Figure 6. The presynaptic axon is at the top of the figure and the postsynaptic dendrite at the bottom. At this very high resolution the unit membranes of the axon (a to b) and the dendrite (c to d) are clearly seen as is the synaptic cleft (b to c) which measures about 200Å. Each unit membrane consists of two dense lines (a and b or c and d) approximately 20 to 25Å thick, separated by a clear zone about 30Å wide. These are clearly the unit membranes which Robertson has shown to be a general feature of cell membranes (15). It is of interest that the unit membranes can be still demonstrated following the rather harsh treatment to which the tissues were subjected during the staining procedures. Note that the cholinesterase stain is located in both the presynaptic and postsynaptic processes with perhaps somewhat more intense staining in the postsynaptic dendrite, and that the staining is limited to the inner component of the unit membrane in each case. As in the previous figure, Figure 5 demonsrates several axons containing "synaptic vesicles" and therefore near termination. However, they have not been stained. Figure 7 represents a section through a region in the cortex containing at least six axo-somatic synapses and stained in the same manner as the previous section. The axons terminating on the cell body in the right half of the figure contain synaptic vesicles (V). However, note that the appositional membranes in the *axo-somatic* synapses are unstained whereas in the three regions designated by the arrows, the *axo-dendritic* synapses are densely stained. This fortuitous section provides an excellent control situation. The insert Figure 8 demonstrates the ultrastructure of the appositional membranes in an area such as that designated by the boxed portion in Figure 7. Again, the unit membranes are apparent in the synaptic endings (see above). Particularly conspicuous is the paucity of staining response at this type of synapse, which has been characteristic of *axo-somatic* synapses.

DISCUSSION

Examination of the mammalian cerebral cortex with the electron microscope has provided a great deal of information concerning the ultrastructure of neurons, neuroglia and their many processes (5 to 10). However, the criteria for the absolute identification of all processes are not always in agreement. This is particularly true in the identification of neuroglial cell types (9, 17), and other cell processes as well. For example, most den-

drites are characterized by their endoplasmic reticulum component. However, other processes are frequently seen which are probably dendrites but which cannot be rigidly proved to be such (13, 14). Thus absolute certainty in classifying all cell processes is not possible.

Some uncertainty exists in identifying synaptic junctions as well. One cannot always be confident that the criteria of synaptic fine structure in one region of the nervous system will hold for another. It has been shown that certain fine structural details are consistently observed at synapses (5, 6) and that they provide a useful morphological classification of nerve terminations. On the other hand, not all processes containing vesicles and demonstrating membrane specialization necessarily constitute a synapse. Strictly spatial definitions lose meaning in interpreting electron micrographs, since all processes are separated by spaces less than 200Å wide.

Obviously, any generalizations concerning the role of "synaptic membranes" in impulse transmission, based upon fine structural specialization, are entirely in the realm of speculation. It must be emphasized that in spite of these objections, a useful classification of the structural events at synaptic junctions is available and has served as the basis for identifying synaptic junctions in this study. Enzymatic specificity has been demonstrated in certain of these regions thereby further supporting the usefulness of this classification.

The large number of axo-dendritic synapses encountered is not surprising when one considers that dendrites comprise approximately 80 to 90 per cent of the surface of the pyramidal cell (18). The greater number of synaptic contacts appear to be situated on dendritic spines, away from the dendritic trunk. Since these are rather small endings and only about one in seventy neurons is stained with Golgi preparations, it is not difficult to understand why they have been difficult to demonstrate with the light microscope. Our results show that synapses on dendritic spines are usually devoid of mitochondria whereas endings on dendritic trunks contain numerous mitochondrial profiles. Whether this represents differences in metabolism in these synaptic regions remains to be seen. Observations on the fine structure of axo-somatic synapses have failed to show any appreciable increased osmiophilia in the appositional synaptic membranes, a feature consistently seen in axo-dendritic synapses. Gray reports occasional thickened regions (14), but Gray's tissues were consistently stained with phosphotungstic acid, whereas our control sections were unstained. Gray's observation may well be due to deposition of the heavy metal stain routinely used in his preparations.

Physiological and pharmacological studies suggest that synapses on dendrites differ functionally from those on the perikaryon (19, 23). Our observations show that following staining procedures designed to demon-

strate acetyl-cholinesterase, an increased osmiophilia or electron density occurs at axo-dendritic synapses. On the other hand, no apparent staining is seen in axo-somatic endings. A most convincing control situation is provided in Figure 7 which demonstrates both types of synapses (see text). It must be inferred that those synapses which stained contain cholinesterase and those that are unstained do not. The reader is referred to the study of Barrnett and Palade (12) for a rigorous discussion concerning the specificity of this reaction. In our hands, cholinesterase inhibitors such as DFP caused inhibition of the reaction.

However, extreme care must be taken in interpreting histochemical results, especially when they are negative. A negative histochemical reaction does not exclude the presence of cholinesterase activity too low to be detected but suggests that the principal role, at least, is not played by acetylcholine. Although there is strong evidence that acetylcholine is a transmitter in widely distributed synapses in the central nervous system, other possible transmitters have also been implicated (20, 21). Our results suggest that cholinesterase is localized in certain axo-dendritic synapses. Denser staining of the postsynaptic processes is observed although presynaptic processes are also stained. The synaptic cleft does not appear to be stained. The possibility that our staining methods may also demonstrate other enzymes as well cannot be entirely excluded. Our observations also suggest that axo-somatic synapses differ in their staining reaction to cholinesterase techniques. A large volume of literature is available on histochemical localization of cholinesterase in the nervous system (22, 23). In general, these studies support our observations. For example, there is a convergence of cholinergic and non-cholinergic fibers on the Purkinje cells in the cerebellum. Cholinesterase-positive granule cell axons make contact with dendrites and cholinesterase-negative basket cell axons with its perikaryon (23).

Recently, Gray and Whittaker (24) have succeeded in isolating an acetylcholine-rich fraction from the brain and have examined this fraction in the electron microscope. Electron micrographs reveal the fraction to consist mainly of processes containing "synaptic vesicles". However, conspicuously present in the fraction are numerous nerve processes with *attached thickened regions* of the *postsynaptic membranes.* In view of our observations it would seem advisable to consider the role of the "synaptic membrane thickening" in ascribing transmitter properties to submicroscopic structures.

In summary, this study has revealed at least two types of synapses in the cortex. These types are distinguishable on the basis of certain ultrastructural specialization in synaptic membranes. Acetyl-cholinesterase staining of these synapses reveals an increased osmiophilia (assumed to be due to

cholinesterase) in certain axon-dendritic synapses and a paucity of staining in axo-somatic synapses. Evidence is presented to support the interpretation that cholinesterase is differentially localized in certain synaptic junctions. It is hoped that further studies of this type will expand our knowledge of synaptic morphology and assist in correlating fine structure with function.

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I wish to thank Professor H. Stanley Bennett for providing us with the Epon embedding techniques developed in his laboratory. Thanks are also due to Mrs. N. U. Kent for her assistance in applying the Epon techniques to brain tissues and to Miss Julia Matthews for her help in developing the Araldite techniques.

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PLATE I

FIG. 1. An electron micrograph through the surface of the cortex demonstrating the many processes which are encountered. A capillary (C) is seen in the middle of the figure and processes derived from the neuroglia cells abut upon the capillary endothelium. Axons, dendrites and glia occupy most of the available space and extracellular spaces are rather small. Approx. × 12,500.

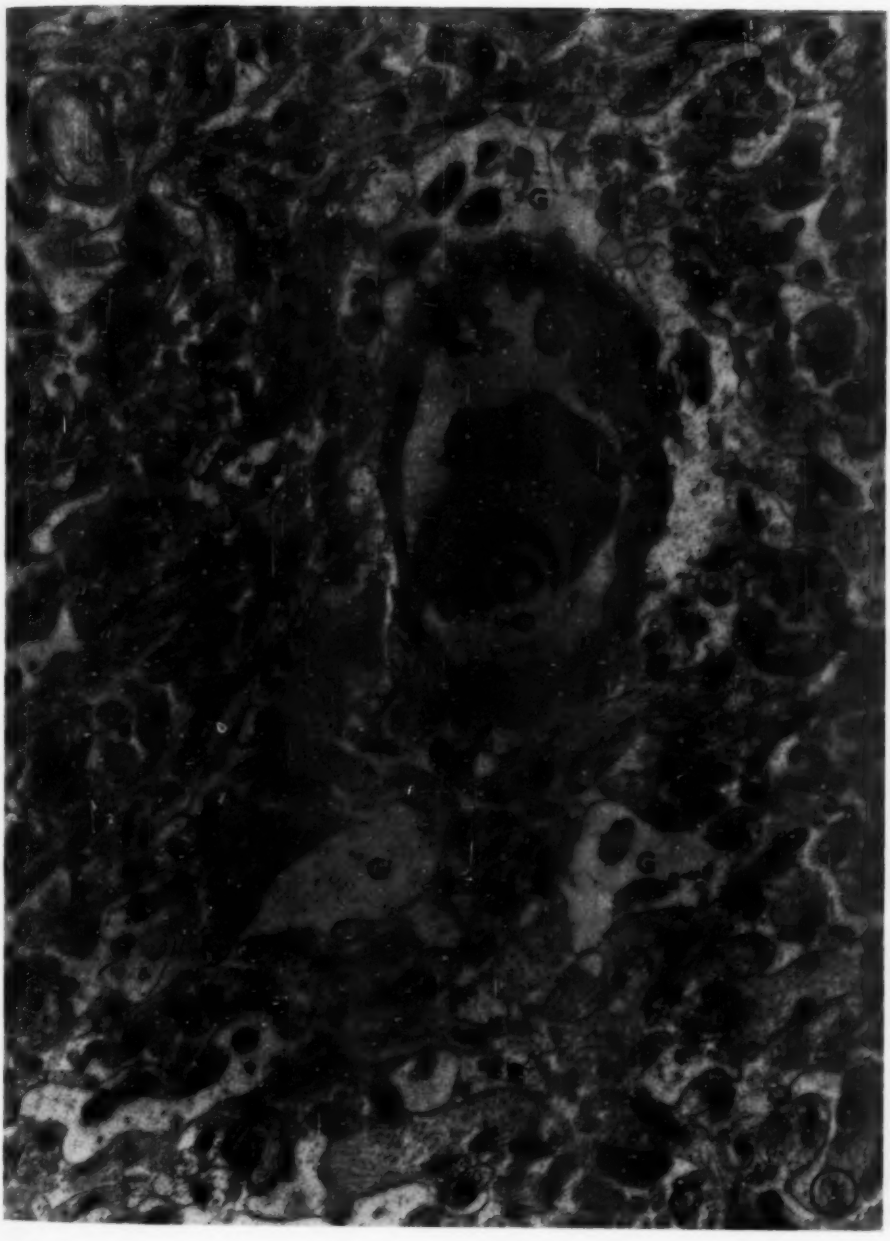
G

PLATE II

FIG. 2. A section through a region of the cortex containing large neurons (N) again demonstrating the close contiguity of membranes (approximately 150Å), see arrows. Myelinated nerve fibers (M) are frequently encountered. Approx. × 17,000.

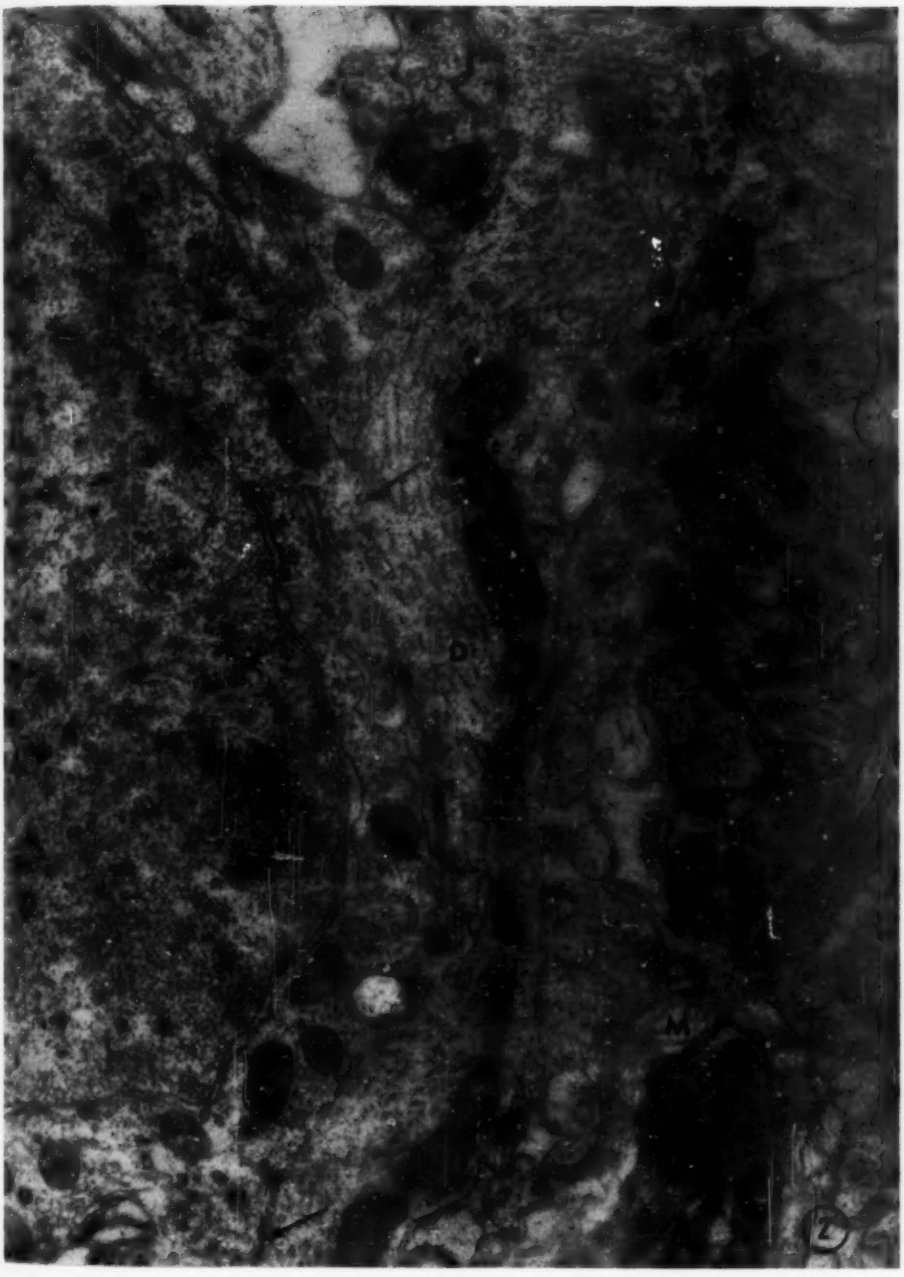
D
M
L

PLATE III

FIG. 3. This micrograph represents a section through region of apical dendrites (D) demonstrating at least six axo-dendritic synapses (see arrows). The presynaptic axons (A) contain clusters of "synaptic vesicles" and the dendrites contain components of the endoplasmic reticulum. The appositional synaptic membranes demonstrate the characteristic thickening seen in this type of synapse. Pale neuroglial processes (G) are apparent but are never seen to reside in or between the synaptic membranes. The synaptic cleft measures about 200Å. Approx. × 16,200.

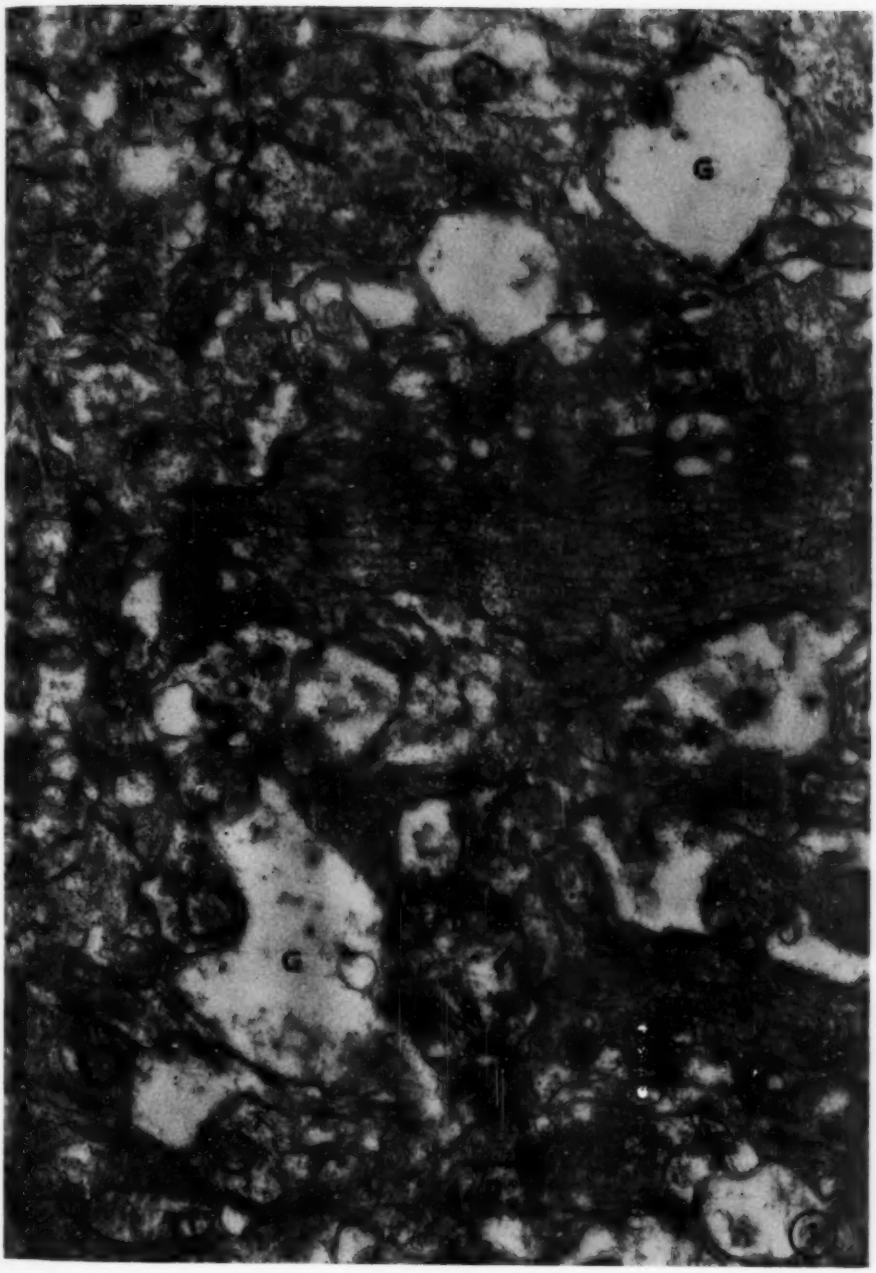
G
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PLATE IV

FIG. 4. Demonstrates a section through apical dendrites containing axo-dendritic endings. This tissue was stained to demonstrate cholinesterase and it is apparent in the regions designated by the X labelled arrows, that an increased osmiophilia all but obliterates the synapse. Presynaptic axons containing "synaptic vesicles" (V) and dendrites (D) are cleary identifiable, however. Small myelinated fibers (M), frequently less than 0.1 micron in diameter are seen. Approx. × 20,000.

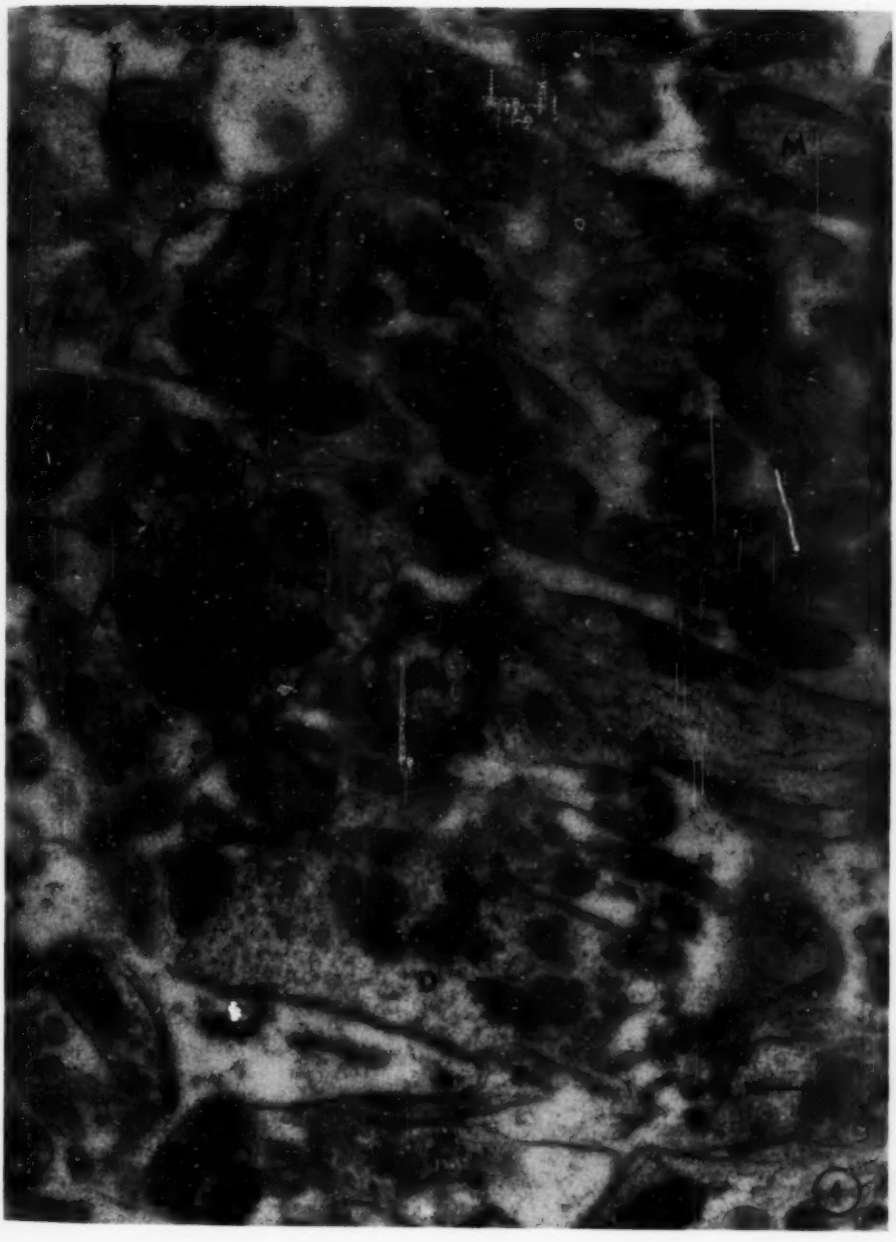

PLATE V

FIG. 5. This is an additional region containing clearly identifiable axo-dendritic synapses, which has been stained by the above procedures. At the locations designated by the arrows, certain axo-dendritic synapses have been intensely stained, representing the localization of cholinesterases in these endings. Note however that not all of the axo-dendritic synapses are stained. Approx. × 47,500.

FIG. 6. The ultrastructure of a stained region such as shown in the previous figure is demonstrated in this electron micrograph. Note that both the pre- and postsynaptic processes are stained (axon above and dendrite below) and that the unit membranes of the region are clearly visible. The unit membranes a to b are those of the presynaptic axon and c to d of the postsynaptic dendrite. The synaptic cleft is clearly seen (b to c). It is of interest that with this particular staining technique, the stain is localized to the inner component of the unit membranes. For further details, see text. × 190,000.

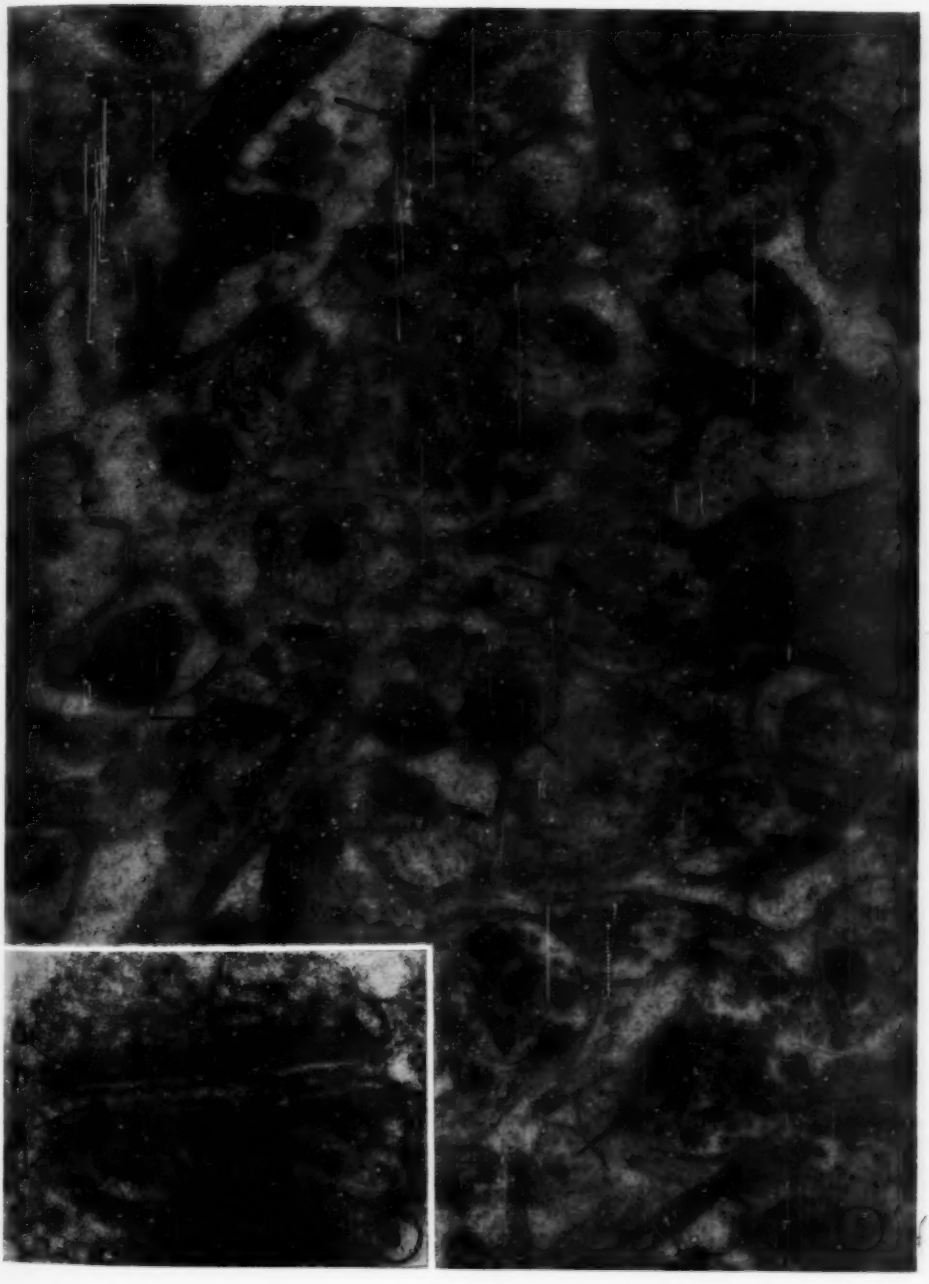

PLATE VI

FIG. 7. This is a particularly informative section through a region in the cortex which demonstrates both axo-dendritic (arrows) and axo-somatic synapses. This tissue was stained to demonstrate cholinesterase. Note the intense staining of the axo-dendritic synapses (arrows) and the paucity of staining in the axo-somatic synapses. The presynaptic processes ending on the perikaryon contain "synaptic vesicles" (V) characteristic of a synapse. At least six synapses on the perikaryon are identified. None of the axo-somatic synapses appear to demonstrate cholinesterase activity. This fortuitous section provides a convincing control situation. Approx. × 19,300.

FIG. 8. The region designated in the previous figure by the box, is shown at very high magnification in Figure 8. Note the conspicuous lack of staining and compare with Figure 6. The unit membranes are again resolved as is the synaptic cleft. See explanation of Figure 6 for details of the fine structure of the synaptic membranes. × 200,000.

PROGRESSIVE RISE IN PULMONARY VASCULAR RESISTANCE IN A PATIENT WITH PATENT DUCTUS ARTERIOSUS. CASE REPORT[1]

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A large patent ductus arteriosus is frequently associated with a high pulmonary vascular resistance and correspondingly severe lesions in the small pulmonary vessels (1). Large numbers of individuals have been studied at all ages and with all grades of severity of vascular disease, and from these findings a composite view has been formed to describe the natural course of the pulmonary vascular disease during the life span of one individual (2). The experimental production of a similar pulmonary vascular disease in animals (3, 4), and its occasional development following anastomotic operations in cyanotic children (5), offer some support to this theory, but case reports containing the crucial evidence—that of individual patients observed to pass through different stages of their disease—have been remarkably rare. Moreover the opportunity for such observations will diminish in the future as surgical correction of the underlying cardiac lesion is now undertaken more readily, and at an earlier age.

This report describes a boy with a large patent ductus arteriosus in whom the pulmonary vascular resistance was observed to increase gradually over eight years, causing the development of equal pressures in the pulmonary and systemic arteries, reduction in the left-to-right shunt, and disappearance of the characteristic continuous murmur.

CASE REPORT

The patient was a white male first seen in 1950 at the age of 18 years. His infancy and childhood were uneventful, with no serious illness. A heart murmur had been heard at the age of 6 years, but no restrictions were imposed, and he had led a normal life, competing favorably in games with other children. His physical and mental development, however, were retarded.

His presenting symptom at the age of eighteen was nocturnal palpitation; he denied undue fatigue and dyspnoea, and gave no history of recurrent respiratory tract infections, cyanosis or syncopal attacks. There was no unusual family history.

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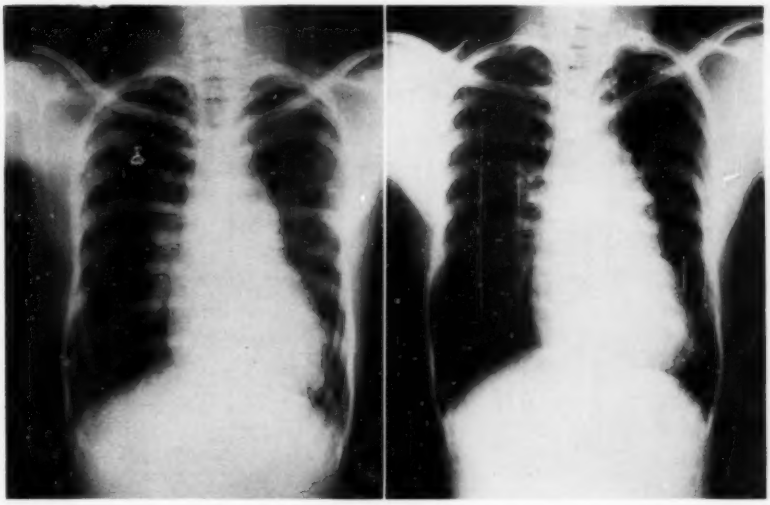

FIG. 1. Chest roentgenograms taken in 1951, one year after ligation and recanalization of the ductus (*left*), and in 1957 (*right*), showing the diminution in peripheral pulmonary vasculature over this period.

On examination he was physically small, but normally proportioned (weight 79 pounds, height 59 inches). There were no external congenital defects. No cyanosis or clubbing was present. The blood pressure was 130/65. The apical impulse was in the anterior axillary line, and there was a moderate systolic lift over the pulmonary area. A loud continuous murmur and thrill was maximal in the second and third left intercostal spaces.

Roentgenograms of the chest showed left ventricular enlargement, dilatation of the main pulmonary artery, and increased peripheral pulmonary vasculature (Fig. 1). The electrocardiogram was within normal limits (Fig. 2). Blood hematocrit was 48 per cent.

A diagnosis of patent ductus arteriosus with large left-to-right shunt was made, and surgical treatment undertaken in August, 1950. A very short ductus, 13 to 14 mms. in diameter, was found. It was closed with purse string sutures of 0 silk at the pulmonary and aortic ends, and one transfixing suture of the same material between.

Recovery was rapid, and no continuous murmur was heard in the immediate post-operative period. Three weeks following operation, a soft continuous murmur had returned, but the arterial pulse pressure was smaller than before operation (B.P. 110/80). Six months later, there was no doubt that the ductus had reopened, a loud classical murmur and wide pulse pressure having returned (B.P. 125/65). Reoperation was suggested but declined by the patient.

The boy was seen regularly from 1950 to 1958, and during this time striking changes were recorded. The classical continuous murmur gradually became fainter, and was last heard in 1955; a short systolic murmur remained along the left sternal border, and by 1957 this was accompanied by a loud systolic ejection click. In 1956 for the first time, a faint decrescendo murmur of pulmonic insufficiency was recorded, and this became progressively louder. The level of arterial diastolic pressure (by cuff) gradually rose; the blood pressure was 105/75 in 1953, and 120/90 in 1957. The chest roentgenogram altered

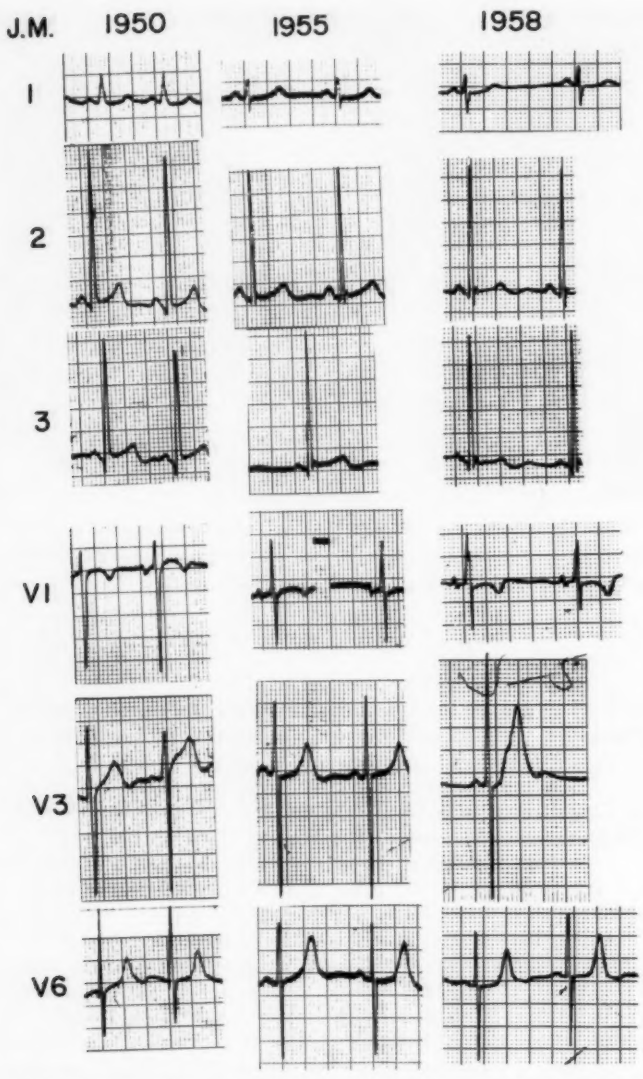

FIG. 2. Serial electrocardiograms showing a progressive increase in right ventricular hypertrophy.

relatively little, but by 1957 there was increased prominence of the main pulmonary artery, with a relative diminution of the peripheral lung vessels (Fig. 1). The electrocardiogram showed increasing right ventricular hypertrophy (Fig. 2). The blood hematocrit rose to 50 per cent in 1956, and to 52 per cent in 1958.

TABLE I

The Results of Cardiac Catheterization Performed in 1953 and in 1958. Oxygen Consumptions Were Not Measured. Note the Equalization of Pressures in Pulmonary and Systemic Arteries, and Reduction of Left to Right Shunt in 1958

	1953			1958		
	Pressure (mm. Hg)	O_2 content (vol. %)	O_2 satn. (%)	Pressure (mm. Hg)	O_2 content (vol. %)	O_2 satn. (%)
S.V.C.	—	16.0	78	—	16.5	75
I.V.C.	—	15.1	74	—	12.4	56
R.A.	−3	16.2	78	4	15.4	70
R.V.	86/−4	16.1	78	106/0	15.3	69
P.A.	90/40	18.5	90	106/55	17.5	79
F.A.	140/64	20.0	97	—	—	—
Aorta	—	—	—	104/53	21.4	97
PBF/SBF*		2.6			1.6	
PVR/SVR**		0.25			0.64	
O_2 capac.		20.5			22.1	

* Ratio of pulmonary to systemic blood flow.

** Ratio of pulmonary to systemic vascular resistance.

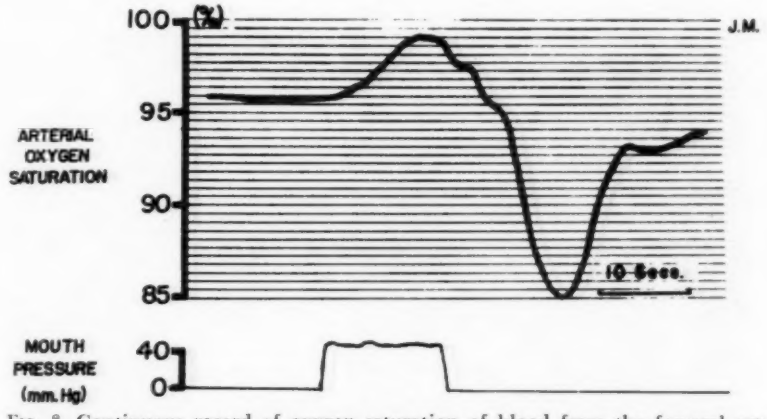

FIG. 3. Continuous record of oxygen saturation of blood from the femoral artery during performance of Valsalva's maneuver in 1958. The marked drop in saturation after release of mouth pressure represents right to left flow through the ductus.

He underwent cardiac catheterization in 1953, and again in 1958, with the results shown in Table I. On both occasions there was a left-to-right shunt through the ductus, but this had diminished considerably in the five year interval, and pressures in the pulmonary artery and aorta had become equal. There was no right-to-left shunt detected on either occasion, but in 1958 changes in the oxygen saturation of blood in the femoral artery indicated free reversal of the shunt following performance of Valsalva's maneuver (Fig. 3).

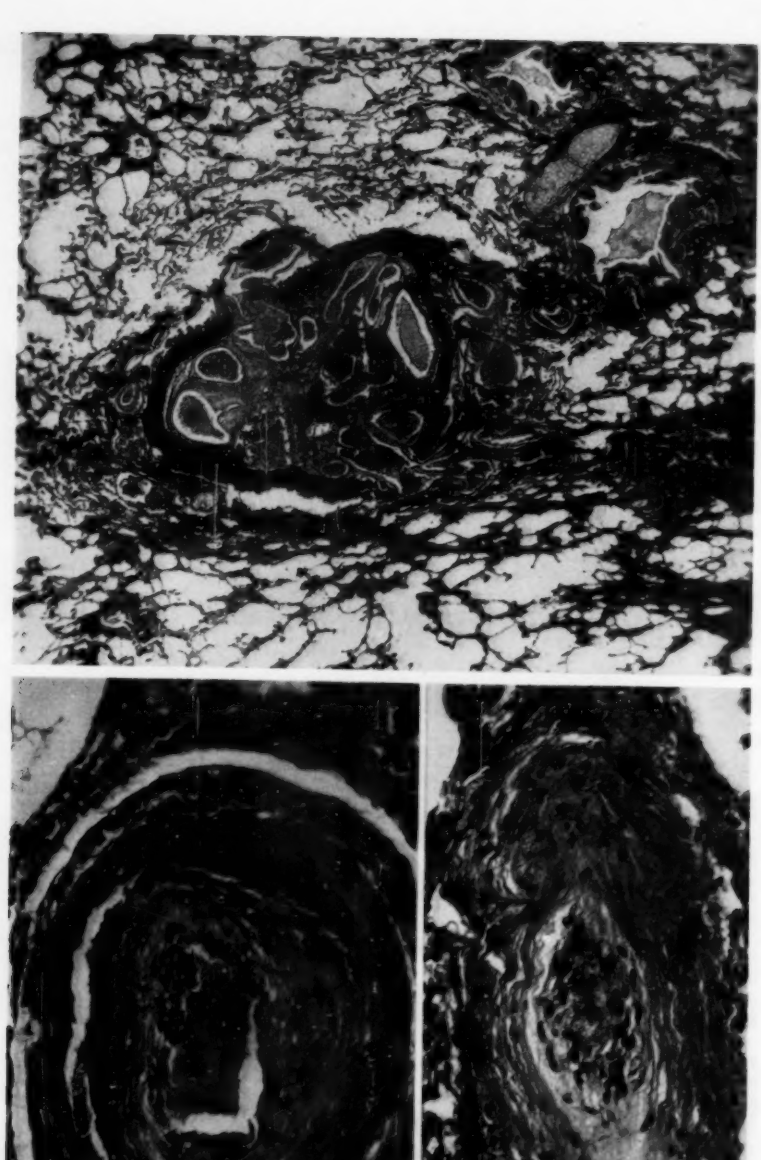

FIG. 4.

His effort tolerance diminished progressively from 1955, and respiratory infections became recurrent and troublesome, though not severe. After cardiac catheterization in 1958, agreement was finally reached that reclosure of the ductus should be attempted, despite an obviously high operative risk.

Operation was performed in April 1958, with the patient cooled to 31 to 32°C. A partially calcified false saccular aneurysm about 15 mms. in diameter was found on the anterolateral surface of the recanalized ductus. No trace was found of the silk ligatures. The pulmonary end of the recanalized ductus was occluded along with the aorta proximal and distal; the ductus was divided and the aneurysm excised. The aortic end was sutured. When the aorta was released after 15 minutes of occlusion, blood pressure fell, and this was aggravated by moderate bleeding. Heart action became weak, venous return poor, and efforts to restore satisfactory circulation were in vain.

Examination at autopsy showed a markedly hypertrophied right ventricle. No abnormalities of the systemic or pulmonary venous drainage were present, the foramen ovale was sealed, and there was no defect of the interventricular septum. The pulmonic valve appeared normal, but the main pulmonary artery was strikingly dilated, and the right branch was approximately twice the diameter of the left. It was not possible to reconstruct the lumen of the ductus arteriosus. The mitral valve was normal, and the left ventricle moderately hypertrophied. The aortic valve was bicuspid, with fused right coronary and non-coronary cusps. The coronary arteries arose and were distributed normally. The proximal aorta was significantly dilated, but beyond the origin of the ductus, the diameter of the vessel was normal. Atheroma was more marked in the arch than in the descending portion of the aorta. The left lung was moderately atelectatic, a consequence of the operative procedure. Sections through the lung parenchyma showed a very prominent vascular tree.

Microscopic examination of the lungs[5] showed abundant evidence of vascular disease. The lumina of many small and some medium-sized vessels were greatly narrowed (Fig. 4), sometimes to the point of obliteration. Endothelial cells appeared large, and protruded into the lumen. The media was hyaline in places, but there was little evidence of necrosis, and no marked inflammatory response; no eosinophils were present. The upper plate in Figure 4 shows a larger vessel with many small channels enclosed within its elastic lamellae; the smaller vessels enclosed within the large show no elastic component in their walls. Sections of the aorta and pulmonary artery were indistinguishable, both demonstrating what has been called the "aortic pattern" of the elastic lamellae (6).

In the lungs, there were several small foci of scarring which contained foreign body giant cells. In addition, there was a peribronchial lymph node in which were found caseous tubercles with acid-fast bacilli. A careful search for further evidence of tubercu-

[5] The authors thank Dr. J. E. Edwards for his kindness in reviewing the lung sections.

FIG. 4. *Top:* Light microphotograph (× 15) of medium-sized pulmonary artery stained with Verhoeff-Van Gieson stain for elastic tissue. The elastic lamellae of the large vessel are intact except at two points. The lumen of the vessel has been replaced by many small channels, the walls of which have no elastic component. *Lower left:* Light microphotograph (× 150) of a small pulmonary artery stained with haematoxylin and eosin. This small vessel shows marked narrowing of its lumen and an unusual hyaline appearance of the media. *Lower right:* Light microphotograph (× 150) of another small pulmonary artery stained with haematoxylin and eosin. The lumen is completely obstructed.

losis revealed only a few tubercles on the epicardium. This tuberculous disease was not considered extensive enough to have contributed in any way to the patient's symptoms or course.

DISCUSSION

The absence of symptoms during infancy, the lack of gross cardiac enlargement, and the similar patterns of elastic tissue found in the pulmonary artery and aorta all suggest that in this patient the pulmonary vascular resistance never fell completely to normal after birth. At the age of eighteen, however, the clinical and operative findings were clearly those of a large left-to-right shunt, and therefore a relatively low pulmonary resistance. Progressively over the following eight years, there was unequivocal reduction of the left-to-right shunt, with disappearance of the continuous murmur and increasing right ventricular hypertrophy. In the third year of this period, the ratio of pulmonary to systemic vascular resistance was 0.25, and by the eighth year this ratio had risen to 0.64. Subsequent microscopic examinations of the lungs indicated that these hemodynamic changes had resulted from obstructive lesions in the small pulmonary vessels.

Out of the wealth of publications concerning patent ductus arteriosus, we have found reports of only six other patients in whom a continuous murmur apparently disappeared as a result of progressive pulmonary vascular disease (7 to 12). In these six, there is reasonable doubt that a continuous murmur was ever present in one (10).

The rarity of such observations is of considerable interest in the light of current concepts of the etiology of pulmonary vascular disease (1, 2, 13). Many patients show both increased pressure and flow in the pulmonary artery in association with patent ductus arteriosus, and a proportion of these should, by these concepts, show a progressive rise in pulmonary vascular resistance. Interruption of the natural history of the condition by surgical treatment, although widely practised in recent years, seems unlikely to explain why this rise has been observed so rarely. As already mentioned, there is at least some doubt that progressive pulmonary vascular disease did occur in one of the six patients previously reported (10). In two others (8, 9), a febrile illness, presumably subacute bacterial endocarditis, occurred during the interval over which the continuous murmur disappeared; in the present case, ligation and recanalization of the ductus preceded the progressive changes observed in the pulmonary vasculature. These features suggest that multiple pulmonary emboli might have played a role in the progressive vascular disease seen in these patients, either in addition to, or in place of, continued exposure of the pulmonary vessels to high pressure and flow.

SUMMARY

A case is reported of a boy with patent ductus arteriosus in whom slowly progressive pulmonary vascular disease led to the disappearance of signs of a left to right shunt.

The rarity of such an occurrence does not seem adequately explained by present concepts of the etiology of pulmonary vascular disease.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY, FEBRUARY 13, 1961

CLINICAL AND PATHOLOGICAL FEATURES OF HYPOXIA IN NEWBORN INFANTS AND YOUNG CHILDREN

FRANK B. WALSH AND RICHARD LINDENBERG

This paper was published in the February, 1961 issue of the Bulletin of the Johns Hopkins Hospital.

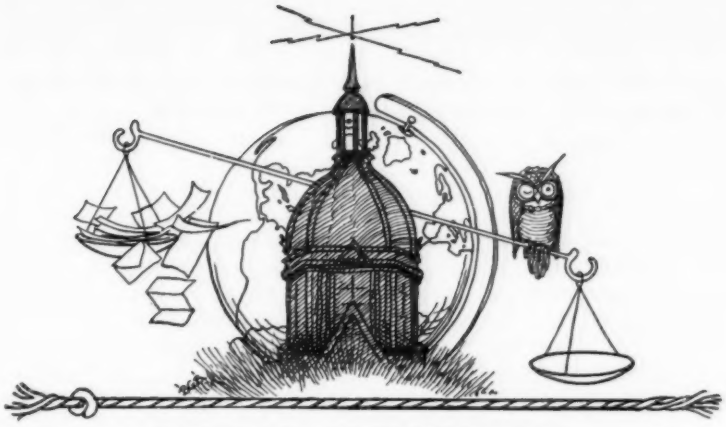
UTERINE MYOMETRIAL ACTIVITY

A. C. BARNES AND D. KUMAR

The mechanism by which the myometrium is maintained in a quiescent state during pregnancy and then becomes activated during labor is not well established. The water, electrolytes and resting membrane potentials were analyzed in the human myometrium since proper ionic and biophysical gradient is an essential prerequisite for the muscle contraction. Twenty-four specimens were obtained from the myometrium overlying the placental and the antiplacental sites during various stages of pregnancy with the idea that placental progesterone might be exerting a local effect on the overlying myometrium. The clinical episodes included therapeutic abortion, elective cesarean section and cesarean hysterectomies. Total tissue K^+ expressed in mEq./kg. wet or dry weight was higher at the placental than at the antiplacental site up to around 38 weeks of gestation. After 38½ weeks of gestation, until term, this difference seems to disappear or K^+ concentration is somewhat lower at the placental site. One biopsy from a three-day postpartum uterus showed no difference at these two sites. Total tissue Cl^-, Ca^{++} and Na^+ levels reveal just the opposite trend when compared with the K^+ trend. The water content at these two sites does not show significant difference.

The higher values of resting potentials, measured by external electrode procedure, at the placental site in all of the five cases around 38 weeks, are concomitant with the total tissue potassium rise except in one case. It is assumed that this might reflect a rise in the intracellular K^+ concentration. In two cases at term the resting potential and the K^+ concentration were insignificant.

It is interesting to note here that the same differences have been reported by other workers by administering progesterone in the estrogen dominated cat uterus.



BOOK REVIEWS

Light Coagulation. By GERD MEYER-SCHWICKERATH. Translated by Stephen M. Drance. 114 pp., $9.50. The C. V. Mosby Co., St. Louis, Mo., 1960.

This book is of primary interest to ophthalmologists, especially those concerned with the fields of retinal detachments and ocular fundus pathology. However, because of the application of this technique in the treatment of certain vascular abnormalities of the retina and the treatment of cerain tumors such as retinoblastomas of children's eyes it is of general interest to those in pediatrics, radiology and internal medicine.

The basis for the clinical application of light coagulation by Meyer-Schwickerath was the observation of solar burns of the retina which produce the well known entity of eclipse blindness. Thus the retinal burns from the solar light source produced scars in the retina quite similar to those produced by diathermy coagulation. The result of his experiments has led to the production of the commercially available Zeiss light coagulator. This apparatus houses a Xenon tube as light source and is optically constructed to direct the high intensity light beam onto the retina through an ophthalmoscope, which permits observation of the coagulation as it is produced.

The first part of the book deals with the historical and experimental background of of the radiation damage to the retina and a description of the light coagulator and directions for its use. The remainder of the book describes the indications for light coagulation which are primarily the treatment of retinal holes without detachment, the treatment of vascular abnormalities of the fundus, and various tumors of the fundus. The book is well illustrated with black and white fundus photographs as well as one page of color illustrations.

The obvious advantage of light coagulation is the ability to treat certain ocular conditions without operative intervention. It must be emphasized that light coagulation has many limitations and is not a panacea for the treatment of any ocular condition. Its use is often as tedious and time consuming as the correspoding diathermy operation although in certain cases it is of decided advantage.

It should be remembered by the reader that many of the uses of light coagulation are of an experimental nature and not necessarily valid forms of therapy. Such an example is the treatment of malignant melanomas of the choroid.

In essence the book is a most interesting monograph on an ingenious new technique which is proving to be of definite clinical value in ophthalmology.

ROBERT B. WELCH

Infectious Diseases of Children, 2nd edition. By SAUL KRUGMAN AND ROBERT WARD. 398 pp., $13.00. The C. V. Mosby Co., St. Louis, Mo., 1960.

At a time when the number of infectious illnesses which can be etiologically defined has been exploded by virologic research, Doctors Krugman and Ward have rendered significant service by offering in textbook form clear and concise descriptions of many of the common infectious diseases of childhood. They have even, as in their own words they state, attempted to bring order to an area of etiologic chaos, the acute respiratory infections. Their attempt is unusually successful. Dr. S. Fradin's schematic diagrams and drawings of the exanthemas are very useful diagnostic aids. But neither of these is the best aspect of the book.

Its unique contribution is the combination of excellent clinical description and intelligent selective review of recent investigative work. It is an outstanding feature. There are of course some deficiencies. ECHO virus exanthemas are not sufficiently compared in a differential manner with other childhood exanthemas. The authors have concerned themselves much more with viral than with bacterial or fungal infections. But this was not intended as an all inclusive text. It is only that when we get so much we come to expect more.

DONALD N. MEDEARIS, JR.

Introduction to Medical Physics. By J. TRYGVE JENSEN. 240 pp., $2.90. The J. B. Lippincott Co., Philadelphia, Pa., 1960.

This paper-bound book might more properly be titled "Physics for Nurses" as it is largely this group to which it is addressed, though the author suggests that students in other fields such as optometry, physical therapy, and x-ray might also find it useful. Unless a course in physics is included in the nursing school curriculum, the book would not be useful as a text, but it would serve admirably as a teaching aid for instructors in many nursing courses, and as a reference for both student and graduate nurses.

The author's seven years of experience in teaching principles of physics to nurses led to the development of this book. His aim seems to be to present basic scientific concepts in a form which is readable, interesting, and not too technical and, in large measure, he succeeds in doing this. He stresses the application of basic principles of physics in diagnostic, therapeutic, and supportive medical and nursing procedures. Included, of course, are such topics as atmospheric pressure, gas laws, factors affecting fluid flow, and so on, the application of which underlie so many nursing activities. Newer material of interest and concern, such as the use of radioactive isotopes and the management of radioactive materials is also included.

The format is good; there is a table of contents, a fairly detailed index, and there are numerous excellent diagrams and illustrations. Aids for further study or for teaching include questions at the end of most chapters, suggestions for the conduct of experiments and demonstrations, and a bibliography for further reading which includes references from both general and nursing literature.

Altogether, this would seem to be a most useful book to have around if one is engaged either in nursing practice as a student or graduate, or in the teaching of nursing.

MARGARET E. COURTNEY

Surgical Diseases of the Pancreas. By JOHN M. HOWARD AND GEORGE L. JORDAN. 607 pp., $20.00. J. B. Lippincott Co., Philadelphia, Pa., 1960.

This volume is a very detailed effort by the authors with the aid of nine able contributors. Included among these is Dr. Allen O. Whipple who has contributed so much to the field of pancreatic surgery.

The various disorders amenable to surgical treatment are considered. Initially important basic facts are presented, including the surgical anatomy, embryology, physiology, and the use of laboratory tests.

Pancreatitis, as might be expected, is considered rather extensively. A classification is presented that differentiates alcoholic and gallstone pancreatitis from some of the other forms. Such a classification is controversial in some quarters, but appears to the reviewer to be useful and logical. Admittedly some cases of pancreatitis are difficult to classify, however, in many cases the direct or indirect cause of pancreatitis is known. A thorough evaluation of the various operative procedures for pancreatitis is available. The conclusions and indications for the procedures are reasonable. One would certainly concur with the statement that the results of surgical management of patients with relapsing pancreatitis have been confusing and disappointing.

Neoplastic diseases of all types are considered in the usual fashion. The radical pancreatico-duodenectomy is not strongly advised for carcinoma of the head of the pancreas, excluding ampullary lesions, as judged by the quoted statistics. This is in agreement with the majority of surgical opinion at this time.

The final portion is devoted to a chapter on subtotal and total resection of the pancreas with particular emphasis on the technical features.

The book is recommended as an excellent effort in this field of medicine and should be very useful as a reference text to medical student, physician and surgeon. The volume is well written and the bibliography is extensive.

WILLIAM F. RIENHOFF, III

Care of the Well Baby. Medical Management of the Child from Birth to Two Years of Age. By KENNETH S. SHEPARD. 224 pp., $3.25. J. B. Lippincott Co., Philadelphia, Pa., 1960.

Dr. Shepard, in his preface to this small but well-organized book, emphasizes that he is presenting "*a way* of approaching the month-to-month problems of mothers and their babies", not "*the way*." The result is a practical text more adaptable to the individual mother, infant, and physician than is seen in the usually dogmatic treatises on this segment of pediatrics.

Part One discusses the newborn period and the handling of the child at each routine examination for the first 24 months. For each visit, there are descriptions of the physical findings, developmental progress, immunization procedures, and sensible suggestions on what to tell the mother. Part Two describes briefly but in a practical way most of the minor and some of the major problems encountered in this age group. Part Three

(Appendix) outlines the hospital procedures, nursing technics, and regulations of a well-run newborn service. In addition to Dr. Shepard, there are nine contributors.

This book will be of particular use to the general practitioner, whose contact with younger patients is limited, and to young pediatricians entering practice, who have had little experience with the handling of normal children in this age group. The experienced pediatrician should already be familiar with and using a program similar to Dr. Shepard's.

JOHN L. PECK

Clinical Orthopaedics, No. 17. Clinical Physiology and Pathology of Bone. Edited by ANTHONY F. DE PALMA. Guest Editor, MARSHALL R. URIST. 387 pp., $7.50 single number. J. B. Lippincott Co., Philadelphia, Pa., 1960.

The publication of this volume in this series of symposium form text represents like most of its predecessors a generally steady increase in thickness and quality. A feature peculiar to this issue is that despite the title, the first section, constituting over four-fifths of the volume, is devoted to aspects of physiology and pathology which most private practitioners are not likely to consider of much contemporary clinical value. The material discussed is largely that of bone tissue analysis, its formation, metabolism, mineralization and destruction. The list of authors is an impressive collection of authorities in these highly technical fields and the articles are written in a sufficiently informal manner as to be generally pleasant, intelligible, and rewarding reading for those orthopaedic surgeons with academic interests. In these latter respects, it is a fitting tribute to Doctor Franklin Chambers McLean in whose honor it has been published and who has done so much to explain the nature of bone and to stimulate others in the frontier of this fascinating field. Like Doctor McLean the numerous authorities in this volume illustrate each from a different approach that a bone can never again be considered merely as a rigid rather inert structural device. It is rather an incredibly complex and animated organ constantly altering its form and consistency in response to numerous stimuli. In a seemingly infinite number of ways it isolates and discharges minute and massive quantities of diverse chemicals as occasioned by the demands of the organism. The symposium form of this volume enables the writers to explain these phenomena in a more penetrating and lucid fashion than the usual journal.

Section two is devoted to general orthopaedics and section three to two case reports. In view of the facts that they are of purely clinical nature and constitute such a small portion of the volume, they seem out of place in the same book as the first section. They might have been better placed in a future volume published for readers with purely clinical interests. Since many copies of this series are purchased singly rather than by subscription this alternative would relieve the orthopaedic surgeon of the necessity of purchasing a book containing the first section in order to obtain the second two sections.

As is always true of this series, the articles are well illustrated and each article has a summary in interlingua. The series has become a routine addition to every well stocked institutional orthopaedic library. This particular issue is also a worthwhile purchase for any individual interested in the more detailed aspects of bone tissue.

GERHARD SCHMEISSER JR.

The Normal Skull. A Roentgen Study. By ROBERT SHAPIRO AND ARNOLD H. JANZEN. 257 pp., $18.00. Paul B. Hoeber, Inc. Medical Book Dept. of Harper & Bros., New York, N. Y., 1960.

This book describes the radiographic anatomy of the normal skull from the viewpoint of radiologists in close cooperation with neurosurgeons.

Among the problems of mutual interest, the borderline between normal and early pathological changes receives particular attention. Accordingly, chapters such as Vascular Supply to the Skull, Sella Turcica, Foramina, Fissures, Canals and Physiological Calcifications are dealt with more extensively. Normal variations of these structures, which could be interpreted as diseased, are shown in x-ray reproductions accompanied by explanatory line drawings. Throughout the entire book, the illustrations are of excellent quality and well explained.

The embryology, the appearance of the skull during the neonatal period and its special features in childhood are discussed in a concise but comprehensive form. One chapter is provided for cranial measurements and their clinical application. It is followed by chapters on Paranasal Sinuses, Ear, Mastoid and Petrous Pyramids. Details on radiographic technique are kept to a minimum.

This publication proves that cooperative efforts between neighboring specialties can be most rewarding. It also recognizes that the distinction between normal variations and early pathological conditions on the roentgenograms is one of the most difficult tasks for the radiologist. Hence, any authoritative contribution of this kind, which attempts to define this borderline, deserves recommendation. Radiologists, neurosurgeons and many general practitioners will find this book very stimulating. To students in this field, it should be most helpful.

MARTIN W. DONNER

The Tonsils and Adenoids in Childhood. By DONALD F. PROCTOR. Edited by MARK M. RAVITCH. 70 pp., $7.50. Charles C Thomas, Publisher, Springfield, Ill., 1960.

This monograph presents in clear logical sequence the historical background of surgery of the tonsil and adenoid, the modern rationale for such surgery, one well developed modern technique of tonsillectomy and adenoidectomy with postoperative care, and a summary of long term results of tonsillectomy and adenoidectomy in children.

The author is uniquely equipped to write this monograph. He has had 25 years experience in otolaryngology in a large medical center. He was professor of anesthesiology in the Johns Hopkins Medical Institutions for a period of four years. He pioneered with Dr. Samuel Crowe, the first Conservation of Hearing Clinic for children in Maryland. Finally for many years he has spent his spare hours working in the graphic arts.

In this publication, the author has drawn freely from his own wide experience. He has also presented a very adequate and useful bibliography. His approach to the surgery of the tonsil and adenoid has been conservative and logical. The thorough way in which he described the dangers of such surgery would justify this book even if it contained no other valuable information.

The subject has been much neglected in the medical literature. The surgery itself has been poorly taught in many places, and it has been done too frequently with very little reason, and, in many instances, without proper preoperative work up or preparation.

Some details of the technique described by Dr. Proctor might be debatable. This reviewer feels that endotracheal tubes in children may prove, at times, more dangerous

than effective, because they give a false sense of security which, may result in carlessness as to the plane of anesthesia in which the child is carried. Likewise, the tube itself may seriously interfere with careful surgery in small throats.

The fact that two million operations are performed in this country each year on the tonsils and adenoids, should be reason for sober reflection as to the necessity for such surgery, and the risks involved in such surgery. These problems have been extremely well covered by Dr. Proctor.

It is the conclusion of this reviewer, that Dr. Proctor's monograph is one that should be kept available in all hospitals where the house staff performs such surgery. This is especially true of those hospitals where full time supervision is not readily available.

JOHN E. BORDLEY

Progress in Experimental Tumor Research. Edited by F. HOMBURGER. 476 pp., $22.00. Published in Basel, Switzerland by S. Karger. Published and distributed in North America By J. B. Lippincott Co., Philadelphia, Pa., 1960.

This volume represents the first in an annual series to be devoted to monographic presentations of various segments of experimental tumor research. Its contents include several discourses on tumor virology by Zilber, Stewart, Graffi, Moore, and their co-authors. Several aspects of chemical carcinogenesis are considered by Boyland, Homburger, Setälä, Scarpelli, and Menkin, with particular emphasis placed on the induction of rodent skin and cervical cancer. Current knowledge of cytotoxic purines and sterols is reviewed by Lettré, and Sokoloff presents many of his own data on oncolytic antibiotics. Le Page and Henderson discuss certain aspects of tumor biochemistry.

On the whole, this series of monographs should prove a useful supplement to those reviews and journals currently presenting material of interest to those engaged in experimental cancer work. Although the reader will question an author's methodology, the pertinence of his data, or the correctness of his interpretations, it would seem that the text is clearly written and appropriately illustrated and referenced.

It is editor Homburger's desire that the contents of future volumes be determined by questionnaires returned from those actively engaged in the field. It will be interesting to observe the future development of this annual, its subject matter, and the quality of its individual presentations.

ALBERT H. OWENS, JR.

Treatment of Cancer and Allied Diseases, 2nd edition. Vol. 4, Tumors of the Breast, Chest and Esophagus. Edited by GEORGE T. PACK AND IRVING M. ARIEL. 667 pp., $30.00. Paul B. Hoeber, Medical Division of Harper & Bros., New York, N. Y., 1960.

Approximately one quarter of all the current cancer deaths in the United States result from neoplasms of the thorax. To label this volume as a surgical text dealing with these common thoracic neoplasms would give no indication of the breadth and depth of the descriptive material included therein. In addition to detailing several current surgical methods for treating cancer of the breast, chest and esophagus and their indications and results, lengthy comment is devoted to the various applications of radiation therapy and mention is made of the role of hormonal therapy in the management of disseminated

mammary cancer. Further, there are discussions of frequently encountered benign lesions and their management, as well as consideration of the pre- and postoperative care of patients undergoing thoracic surgery, their anesthesia, and the anatomic and physiologic derangements which result from their procedures.

Much of the material presented is based on knowledge and technical improvements gained in the twenty-year period since this book's first edition. The authoritative contributions of 67 authors have been carefully organized and correlated. The text itself is set forth in a very clear-cut manner, the illustrations are both numerous and pertinent, and each chapter is accompanied by an extensive reference list. Altogether, this volume should serve well as a comprehensive and conveniently usable single surgical source book.

ALBERT H. OWENS, JR.

Symposium on Glaucoma. Edited by WILLIAM B. CLARK. 314 pp., $13.50. The C. V. Mosby Co., St. Louis, Mo., 1959.

This is the record of the symposium on glaucoma held in New Orleans in 1957. It opened with a discussion of the anatomy and pathology of the eye in relation to glaucoma by Dr. Dvorak Theobald, followed by an excellent and much needed discussion of surgical anatomy by Dr. Kenneth Swan, who later discussed techniques of filtration operations in detail and the miotic treatment of glaucoma. Dr. Bernard Becker contributed sections on the biochemistry of aqueous production, tonography in provocative tests, diamox and other inhibitors of aqueous flow, hypersecretion glaucoma, primary glaucoma and cataract, glaucoma and vein occlusion, glaucoma in the Negro or diabetic and other very pertinent topics.

Dr. Morton Grant, the originator of modern tonography, discussed tonography and aqueous flow. Dr. Joseph S. Haas presented sections on perimetry, diagnostic and provocative tests, clinical manifestations of glaucoma, and surgery for angle closure.

Dr. A. E. Maumenee gave the most complete discussion of medical control I have ever seen. This was set out very clearly with very specific detail. He also discussed surgery for congenital glaucoma. Dr. Lorenz Zimmerman discussed hyaluronidase sensitive acid mucopolysaccharide in the trabecula. Dr. Harold Sheie gave a section on surgery giving exact details of surgical technique used at his clinic.

I found this book very helpful and would recommend it to anyone interested in glaucoma. Generally the book has a clinical orientation. In some places the discussions tend to be misleading, although the necessary points are usually clarified later in the book. For example, the reader might gain the impression that the anterior chamber deepens centrally after iridectomy for closed angle glaucoma, but much later in the book it is brought out that only the extreme peripheral anterior chamber deepens. Again, the fact that there is substantial danger of an acute attack of closed angle glaucoma many hours after the mydiatic test is not brought out until long after the formal discussion of the test. One might be left with the impression that the cribriform plate is bowed backwards in glaucoma. This is now known to occur only after light perception has been lost. Generally the discussions are so specific on controversial points that there is bound to be disagreement over them. This very characteristic, however, adds tremendously to the value of the book.

CARTERET LAWRENCE

BOOKS RECEIVED FOR REVIEW

Allergie-Probleme in Kindesalter. By GERHART ERDMANN. 165 pp., Brosch. DM 14,60. Johann Ambrosius Barth, Leipzig, Germany, 1961.

British Medical Bulletin, Vol. 17. No. 1, January, 1961. Hypothermia and the Effects of Cold. 78 pp., $3.25. Published by the Medical Department. The British Council, London, England.

Cell Physiology of Neoplasia. A Collection of Papers Presented at the Fourteenth Annual Symposium on Fundamental Cancer Research, 1960. Edited by THE STAFF and published for THE UNIVERSITY OF TEXAS M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE. 653 pp., $10.50. University of Texas Press, Austin, Texas, 1961.

The Choice of a Medical Career. Essays on the Field of Medicine. Edited by JOSEPH GARLAND AND JOSEPH STOKES, III. 231 pp., $5.00. J. B. Lippincott Co., Philadelphia, Pa., 1961.

Ciba Foundation Study Group No. 5. Regulation of the Inorganic Ion Content of Cells. In honour of Prof. E. J. CONWAY. Edited by G. E. W. WOLSTENHOLME AND CECILIA M. O'CONNOR. 100 pp., $2.50. Little, Brown & Co., Boston, Mass., 1960.

Cirrhosis of the Liver. By MARTIN S. KLECKNER, JR. Foreword by J. ARNOLD BARGEN AND ARCHIE H. BAGGENSTOSS. 729 pp., $24.50. Charles C Thomas, Publisher, Springfield, Ill., 1960.

Clinical Bacteriology. By E. JOAN STOKES. Foreword by A. A. MILES. 309 pp., $7.00. Edward Arnold, Publishers, London, England, 1960. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

Clinical Toxicology. By C. J. POLSON AND R. N. TATTERSALL. 589 pp., $10.00. J. B. Lippincott Co., Philadelphia, Pa., 1961.

Congenital Malformations of the Heart, revised edition. Volume II, Specific Malformations. By HELEN B. TAUSSIG. 1049 pp., $17.50. Published for The Commonwealth Fund by Harvard University Press, Cambridge, Mass., 1960.

The Crisis in American Medicine. Edited by MARION K. SANDERS. Foreword by JOHN FISCHER. 149 pp., $3.75. Harper & Bros., New York, N. Y., 1961.

Der Schnupfen. Beiträge zur Ätiologie, Pathogenese, Diagnostik und Therapie mit besonderer Berucksichtigung der Allergie. By G. EIGLER AND D. G. R. FINDEISEN. 172 pp., Brosch. DM 10.—; gebunden DM 12. Johann Ambrosius Barth, Leipzig, Germany, 1960.

The Hand. A Manual and Atlas for the General Surgeon. By HENRY C. MARBLE. 207 pp., $7.00. W. B. Saunders Co., Philadelphia, Pa., 1960.

Instructional Course Lectures 1960. Vol. 17. Edited by FRED C. REYNOLDS. 421 pp., $18.50. The C. V. Mosby Co., St. Louis, Mo., 1961.

Medical-Surgical Nursing, 2nd edition. By KATHLEEN SHAFER, JANET SAWYER, AUDREY MCCLUSKEY AND EDNA BECK. 876 pp., $8.75. The C. V. Mosby Co., St. Louis, Mo., 1961.

EVIDENCE FOR THE CENTRAL MEDIATION OF SECONDARY PERISTALSIS IN THE ESOPHAGUS[1]

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Over fifty years ago Meltzer (1, 2) classified esophageal peristalsis according to its mode of initiation. As defined, primary peristalsis is initiated by swallowing, and secondary peristalsis is the response to a local stimulus, such as a bolus in the esophagus. The role of secondary peristalsis in normal esophageal function is believed to be the transport either of material left in the esophagus after a primary peristaltic wave or of material introduced by gastroesophageal reflux. Thus, primary and secondary peristalsis differ by definition in terms of their method of elicitation. Meltzer regarded primary and secondary esophageal peristalsis as different not merely in terms of their mode of initiation but also in terms of the neural pathways involved. Thus, it was postulated that primary peristalsis is centrally controlled and once initiated is independent of local reflexes, whereas secondary peristalsis is mediated by local reflexes with the afferent stimuli released continuously from the esophageal wall as it is sequentially stimulated by a moving bolus. This formulation of the mechanisms of primary and secondary peristalsis has remained widely accepted until recent years.

Recent studies (3, 4, 5, 8) have shown similarities between primary and secondary peristalsis. To further delineate the relationship, simultaneous recordings of motor activity above and below the site of esophageal stimulation have been studied and compared with peristalsis following a swallow. The results show further similarities between primary and secondary peristalsis and suggest a central pathway for the mediation of secondary peristalsis.

METHODS AND MATERIALS

These studies were performed on ten normal volunteers from whom no history of esophageal disease or symptomatology could be elicited. Tube assemblies were constructed containing a total of five polyvinyl catheters. To one of the tips a 35 ml. capacity balloon was attached. The balloons and tips were varied in location so that one to four tips could be positioned above the balloon (see Fig. 1). The open-tipped water-

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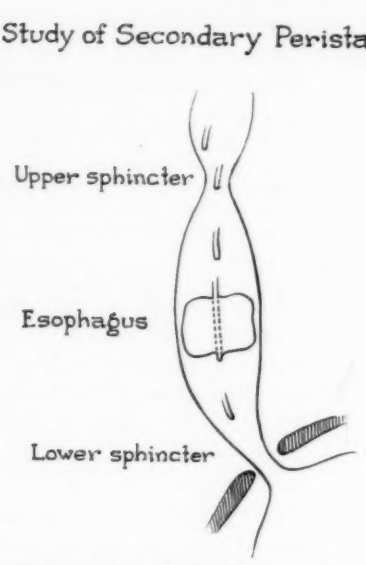

FIG. 1. The above diagram schematically represents the experimental setup for the study of secondary peristalsis. The positions of the distending balloon and the four recording tips could be altered independently so that one to four recording tips could be placed above the balloon. By varying the balloon position from lower to upper esophagus, pressure recordings were obtained as described in the text.

filled catheters were connected to transducers with simultaneous four-channel recording.[4] The recordings were made with the subjects supine and the zero point of the transducers leveled at the posterior axillary line. Intraluminal pressure was recorded at numerous levels from lower esophageal sphincter to pharynx. At each level the esophageal motor response to swallowing was compared to that following balloon distension. The subjects were observed constantly to make certain that the esophageal motor response was secondary peristalsis and not the response to inadvertent swallowing. Only when balloon distension clearly did not lead to swallowing was the response regarded as a secondary peristalsis.

RESULTS

In Figure 2 the sequence of events following a swallow of water was compared to the response to balloon distension. The balloon was positioned in lower esophagus. The recording tips were in the lower esophageal sphincter, 5 cm. above the sphincter, and 5 and 10 cm. above the distending balloon. Following a swallow, the peristaltic wave as manifested by the pressure peak was first observed at the upper recording tip then proceeded distally at a rate of 2 to 4 cm. per second. The relaxation

[4] In these experiments a Sanborn four-channel recorder and Sanborn differential pressure transducers, model #267B were used.

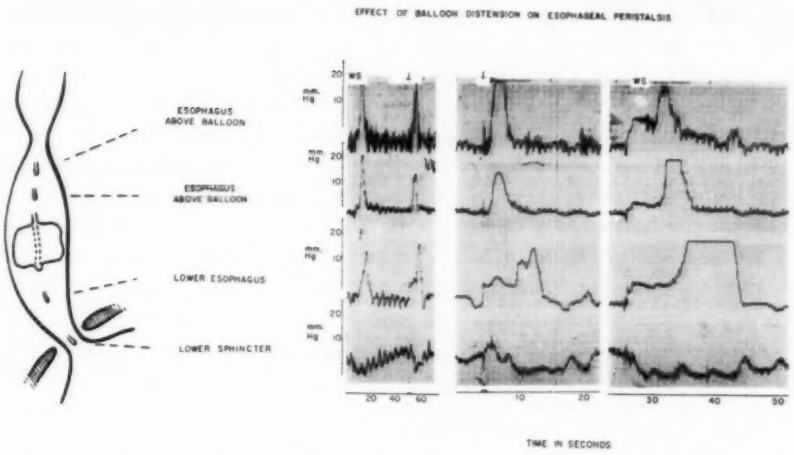

FIG. 2. The above motor recording was obtained with the balloon in the lower esophagus. The two recording tips below the balloon were in lower esophageal sphincter and 5 cm. above the sphincter. The two remaining tips were positioned 5 and 10 cm. above the distending balloon. On the left the motor response to a swallow (indicated by WS) was compared to the response to balloon distension (indicated by the arrow) at a slow recorder speed (1 mm./sec.). Following a swallow, peristalsis as indicated by the peak of the pressure rise was first observed in the upper tip then proceeded down the esophagus. Relaxation of the lower sphincter preceded the arrival of the peristaltic wave. Similarly, following rapid inflation and deflation of a balloon with 15 ml. of air, the same sequence of events was observed. On the right primary (WS) and secondary peristalsis (↓) were recorded at more rapid paper speed (5 mm./sec.).

of the lower esophageal sphincter occurred prior to the arrival of the peristaltic wave. Following the rapid inflation and deflation of the balloon with 15 ml. of air, peristalsis occurred. The peristaltic wave began above the balloon, passed through the segment of esophagus containing the balloon, then continued caudad.

It has been demonstrated previously that esophageal peristalsis will proceed distally from a distending balloon (4, 5, 8). However, as seen in Figure 2, peristalsis begins not at, but above the level of the balloon. Once initiated the peristaltic wave passes progressively in a manner identical with that of primary peristalsis.

In all our studies in which secondary peristalsis was produced by balloon distension the peristaltic wave appeared above the level of the distending balloon. The upper recording tips were then withdrawn stepwise to ascertain at what level secondary peristalsis first appeared. Figure 3 demonstrates the record obtained with the tips positioned in pharynx, upper esophageal sphincter, lower esophagus, and lower sphincter with

the distending balloon in the upper esophagus. Following a swallow, pharyngeal contraction, relaxation of the upper sphincter, a peristaltic wave in the body of the esophagus, and relaxation of the lower esophageal sphincter were observed. When secondary peristalsis was initiated by balloon distension, no pharyngeal peristalsis occurred, but the upper sphincter relaxed and a peristaltic wave followed. Esophageal peristalsis was initiated above the level of the distending balloon regardless of whether the balloon was in the upper (Fig. 3) or lower (Fig. 4) esophagus. Beginning with relaxation of the upper esophageal sphincter, peristalsis in the body of the esophagus and relaxation of the lower esophageal sphincter were identical with that seen following a swallow.

Frequently balloon distension initiated sphincteric relaxation without stimulating peristalsis (Fig. 5), (Table I). This same phenomenon of sphincteric relaxation without an ensuing peristaltic wave is occasionally observed following a swallow as illustrated in Figure 5. Sphincteric opening without peristalsis was observed in 29 (6%) of the 465 swallows recorded (Table II).

In nine of the ten subjects sphincteric opening was followed by eso-

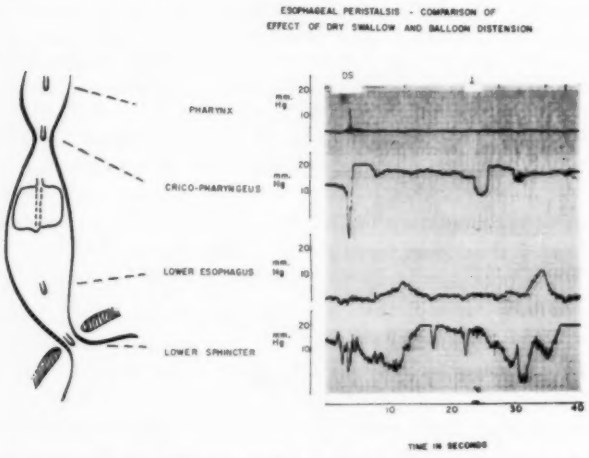

FIG. 3. This recording was obtained with the tips in pharynx, upper sphincter, lower esophagus, and lower sphincter and with the distending balloon in the upper esophagus. The normal sequence of pressure changes following a swallow (indicated by DS) is shown on the left with relaxation of the upper sphincter (cricopharyngeus) preceding pharyngeal peristalsis and relaxation of the lower sphincter preceding esophageal peristalsis. In secondary peristalsis, initiated by a 30 ml. balloon distension, (indicated by ↓), no pharyngeal peristalsis occurred but the remainder of the esophageal motor response mimicked primary peristalsis.

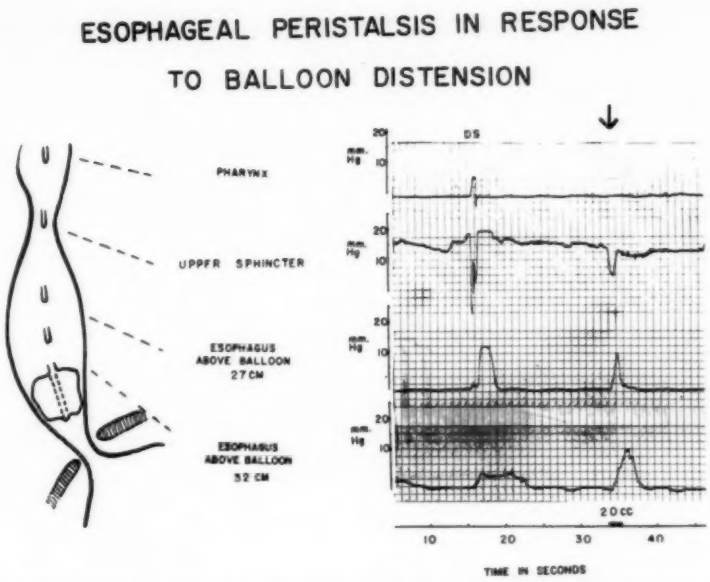

FIG. 4. The above pressure recording was obtained with the distending balloon in the lower esophagus. The recording tips were all placed above the balloon, in pharynx, upper sphincter, and at two points, 5 cm. apart, in mid-esophagus. The motor response to balloon distension indicated by the arrow began with relaxation of the upper sphincter followed by a peristaltic wave which traversed the esophagus. The response to a swallow is on the left for comparison.

phageal peristalsis (Table I). Sphincteric relaxation alone occured following 172 (35%) of the 485 distensions employed. Sphincteric relaxation alone or followed by esophageal peristalsis occurred following 352 (72%) of the 485 distensions. Half of the sphincteric relaxations (51%) were followed by peristalsis in the body of the esophagus.

The optimum balloon volume for initiating sphincteric relaxation and peristalsis varied from 10 to 30 ml. in different individuals. In several of the subjects the larger balloon volumes were more frequently associated with sphincteric relaxation without peristalsis.

In six of the ten subjects balloon distension was occasionally followed by esophageal spasm—synchronous contractions at all points measured (Fig. 6), (Table I). This type of response followed 23 (5%) of the 485 distensions.

To further delineate the role of central versus local neural pathways in the propogation of secondary peristalsis, recordings of primary and secondary peristalsis were obtained on two mongrel dogs (BL-112, BL-113).

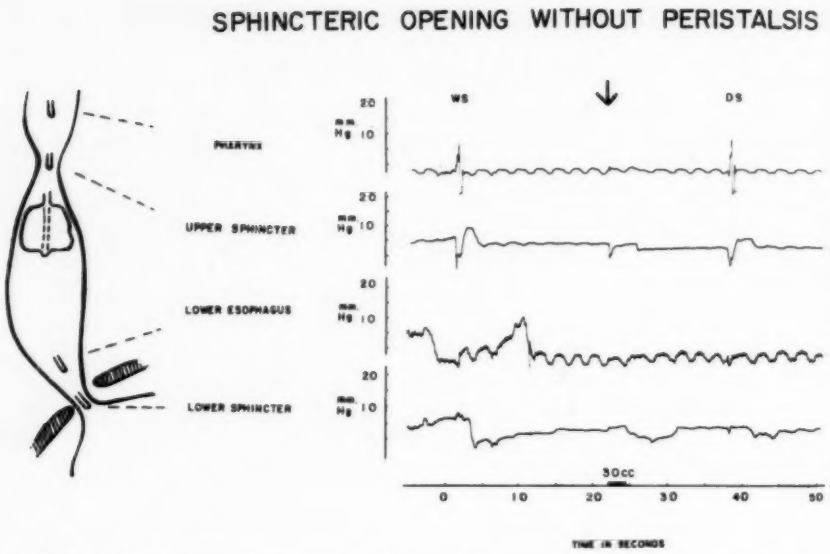

FIG. 5. Relaxation of both upper and lower sphincters without peristalsis is shown following balloon distension indicated by the arrow. This same phenomenon of sphincteric relaxation without peristalsis following a swallow (indicated by DS) is demonstrated on the right. The usual response to a swallow with sphincteric relaxation and peristaltic contraction is shown on the left.

TABLE I

Secondary Peristalsis Produced by Balloon Distension

Subject	Age	Number of Balloon Distensions	Upper Sphincteric Opening		Upper Sphincteric Opening with Peristalsis		Synchronous Contraction	
			Number	%	Number	%	Number	%
DB	24	160	40	25	79	50	5	3
AR	34	102	37	36	38	37	5	5
MS	30	26	7	27	1	4	8	30
NS	25	11	1	9	6	55	0	0
JW	28	52	21	40	13	25	2	4
DS	24	63	33	52	26	41	1	2
BH	26	32	10	31	3	9	2	6
FC	32	31	18	58	12	39	0	0
JB	25	2	2	100	0	0	0	0
GP	29	6	3	50	2	33	0	0
Total		485	172	35%	180	37%	23	5%

TABLE II

Swallows without Ensuing Peristalsis

Subject	Number of Swallows	Swallows followed by Esophageal Peristalsis		Swallows with Sphincteric Opening without Peristalsis	
		Number	%	Number	%
DB	117	116	99	1	1
AR	82	65	79	17	21
MS	23	23	100	0	0
NS	60	60	100	0	0
JW	37	32	86	5	14
DS	69	63	91	6	9
BH	11	11	100	0	0
FC	49	49	100	0	0
JB	0	0	0	0	0
GP	17	17	100	0	0
Total	465	436	94	29	6

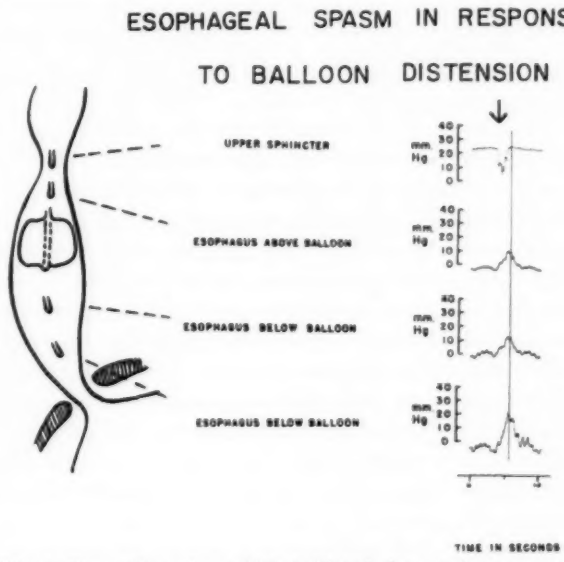

FIG. 6. The balloon distension which initiated the synchronous contraction (non-peristaltic) is indicated by the arrow. The vertical line demonstrates that the peak pressures recorded are simultaneous.

Following rapid inflation and deflation of a balloon with 15 to 30 ml. of air, secondary peristalsis occurred. As observed in man, peristalsis began above the site of stimulation then proceeded caudad. A high cervical esophageal transection (6 cm. below the cricopharyngeus) with immediate

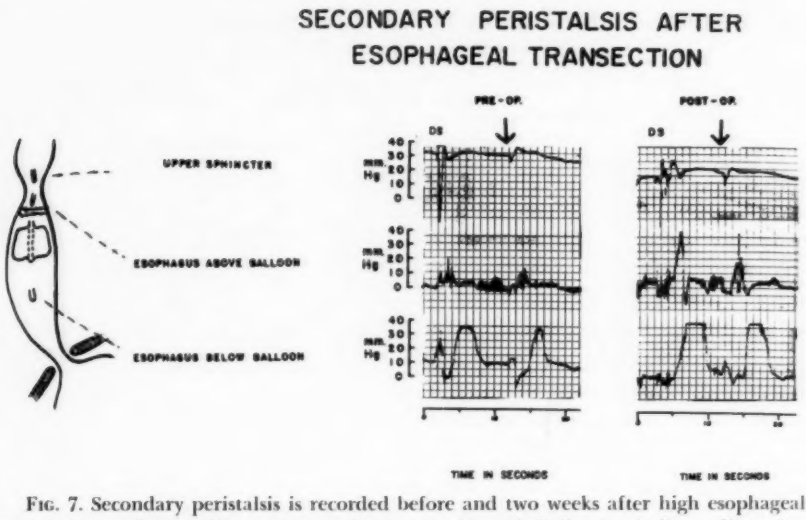

FIG. 7. Secondary peristalsis is recorded before and two weeks after high esophageal transection and immediate anastomosis were performed. Following balloon distension (indicated by the arrow) upper sphincteric opening is followed by a peristaltic contraction which passes down the esophagus. There is no significant difference between preoperative and postoperative responses.

anastomosis was performed. One of the animals (BL-113) became unmanageable postoperatively and satisfactory studies could not be performed. In the second dog (BL-112) pressure recording three to four weeks postoperatively demonstrated that the motor response of the esophagus to balloon distension was unaltered by esophageal transection. Following balloon distension the cricopharyngeus relaxed and peristalsis proceeded down the esophagus as it had preoperatively (See Fig. 7). The appearance of secondary peristalsis above the level of transection in response to a stimulus applied below the transection demonstrates that the interruption of intramural pathways at one point does not prevent secondary peristalsis from traversing the esophagus in a normal fashion and is further evidence to suggest that secondary peristalsis is mediated by extramural neural pathways.

DISCUSSION

By definition primary and secondary peristalsis differ in their mode of initiation; primary peristalsis occurs following a swallow and secondary peristalsis occurs in response to a local stimulus, such as a bolus in the esophagus. Meltzer concluded (1, 2) that primary and secondary peristalsis differed not only in their mode of initiation but also in the neural

pathways involved in their propogation. This conclusion was based principally on two sets of observations. First, Kronecker and Meltzer (10) observed that the lower part of the esophagus as well as the cardia continued to show peristaltic contractions following a swallow even after the ligation or division of the esophagus. Mosso (6) reported similar findings in the body of the esophagus, indicating that the propogation of primary peristalsis was not altered by interruption of intramural pathways. Second, Meltzer observed that secondary peristalsis, in contrast to primary peristalsis, did not progress in the lower esophagus if it had been divided transversely (11). These views were summarized by Meltzer as follows (1) "All the movements of the complicated act of deglutition (primary peristalsis) are managed by a reflex mechanism, with only one sensory stimulus for its intitiation and a series of consecutive motor impulses going to every part of the long path of deglutition; it is practically a single reflex. The reflex mechanism of the secondary peristalsis on the other hand, consists of a chain of reflexes; each part of the esophagus sends up to the center a sensory impulse started by the presence of the bolus in that part and receives in turn a motor impulse. The secondary peristalsis therefore requires the presence of some sort of a bolus within the esophagus and presupposes the integrity of the latter; whereas the primary peristalsis requires neither a bolus nor the integrity of the esophagus."

Other evidence cited in support of the above differentiation between primary and secondary peristalsis included a greater resistance of secondary peristalsis to depression following anesthesia (2) (indicating a local neural mechanism) and abolition of secondary peristalsis by unilateral vagotomy, (a procedure which is said to affect primary peristalsis only minimally if at all).

Evidence available in Meltzer's time was not all in accord with this formulation. Mosso's early experiments (6) indicated that an olive-sized wax pellet would progress beyond the site of esophageal transection in response to a swallow (primary peristalsis). On the other hand, Wild (7) obtained results which did not duplicate those of Mosso. In fact, recent experiments of Hwang (3) showed that secondary peristalsis distal to a distending balloon was able to traverse a segment of anesthetized esophageal mucosa. Moreover, in the dog experiment described above, both primary and secondary peristalsis were observed to begin cephalad to the site of transection, and then to pass caudad through the site of transection. The very fact that balloon distension below the site of esophageal transection initiated peristalsis above the transection requires afferent pathways outside the wall of the esophagus.

In both man and an experimental animal (dog), no qualitative or quantitative differences were observed between primary and secondary peri-

stalsis. Sphincteric relaxation and esophageal peristalsis were observed following 37 per cent of the balloon distensions in this study. Relaxation of the upper sphincter without peristalsis was seen in 35 per cent of the distensions. This phenomenon of sphincteric relaxation without peristalsis is not unique to balloon distension. For swallowing itself is not always followed by a peristaltic wave. In a recent study (12) no esophageal peristalsis followed 25 per cent of the swallows. On the other hand, in the present study (Table II) only 6 per cent of the swallows were not followed by a peristaltic wave. The difference between these two series may well be accounted for by the difference in the age of the subjects. In the current series all of the subjects were young healthy adults. We have observed that the incidence of failure of peristalsis to occur following a swallow increases with age.

Balloon distension initiated peristalsis in 37 per cent of the attempts. In other studies where peristalsis was measured only distal to the distending balloon, peristalsis was triggered following 26 per cent (4) and 47 per cent (5) of the distensions.

The present investigation failed to show any difference between primary and secondary peristalsis in the esophagus. Indeed, from the upper esophageal sphincter caudad, an adequate stimulus, whether it be a swallow (by definition initiating a primary peristalsis) or a local stimulus (by definition initiating a secondary peristalsis) will lead to a response which is the same in all essential features. The implication of these observations is that both primary and secondary peristalsis are mediated via a central (and presumably the same) neural pathway.

CONCLUSION

The manometric features of primary and secondary peristalsis were compared in ten normal subjects. Secondary peristalsis was elicited by inflation of a balloon in both lower and upper esophagus. In all ten subjects balloon distension caused opening of the upper esophageal sphincter and in nine of ten a peristaltic wave followed. This peristaltic wave originated not at the level of the stimulus, but above it at the upper esophageal sphincter, then swept down the esophagus in a fashion identical to that of primary peristalsis. These same observations were made in the dog both before and after high esophageal transection. These findings render untenable the concept that secondary peristalsis is a purely local chain of reflexes initiated and maintained by sequential stimulation of the esophageal wall by a moving bolus.

It appears that the esophageal motor response is the same regardless of the initiating stimulus. These observations suggest that both primary and

secondary peristalsis are mediated through the central nervous system and have the same efferent pathway.

ACKNOWLEDGMENT

The authors wish to thank Mr. Vivien Thomas for his performance of the esophageal transections.

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EXPERIMENTAL POLIOMYELITIS FOLLOWING INTRAMUSCULAR VIRUS INJECTION

I. The Effect of Neural Block on a Neurotropic and a Pantropic Strain[1, 2]

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It is generally agreed that the usual portal of entry in human poliomyelitis is the oropharynx or lower intestinal tract. Although a number of artificial portals have been utilized in experimental studies of pathogenesis, the primary purpose has been elucidation of routes by which virus spreads from the natural sites of primary invasion. The occurrence of a group of human cases of inoculation poliomyelitis, the Cutter incident of 1955, stimulated our interest in the pathogenesis of poliomyelitis following intramuscular virus injection.

In the Cutter incident 65 paralytic cases occurred within 4 weeks following the inoculation of about 400,000 persons (1). These cases showed a high frequency of initial (and most severe) arm involvement (39 or 60%), in contrast with the predominance of leg paralysis usually seen in human poliomyelitis. Further, the predominance of arm paralysis was related to the high frequency of arm inoculation, while leg paralysis was frequent among the smaller number of cases inoculated in the leg. In sum, 46 cases (71%) showed a correlation between sites of inoculation and of first paralysis.

It was clear that any route proposed for virus spread from muscle to central nervous system would have to account for this localization of paralysis. Two mechanisms were postulated, as shown in Figure 1. First, virus might spread by the neural route along neurons innervating the injected muscle, and invade their perikarya before spreading through the spinal cord, thus causing initial paralysis in the inoculated limb. Second, virus might enter the circulation, either directly or via lymphatic channels, and invade the CNS through the blood-brain barrier. Localization could be explained by the provoking effect, in which the injection enhances the permeability of the capillary bed in the corresponding part of the spinal cord.

Previous work in this laboratory has shown that under certain conditions

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[2] We wish to thank Mr. William D. McClaskey for expert technical assistance.

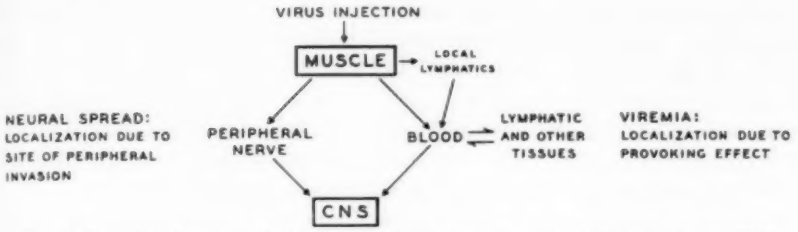

FIG. 1. Postulated routes of virus spread from intramuscular injection site to CNS.

each of the above mechanisms may be operative. If poliomyelitis is induced by dipping the cut end of a peripheral nerve into a virus solution, virus spreads along the nerve trunk to the cord, and initial paralysis includes the corresponding limb (2). If a pantropic virus is introduced directly into the circulation, with subsequent viremia, intramuscular injection of a wide variety of substances will markedly enhance the frequency of involvement of the injected limb (3).

Different poliovirus strains vary widely in their invasive properties, and it appeared likely that these properties might determine the relative importance of various routes of spread. For this reason experiments were conducted with two markedly different strains, both of which were known to be highly paralytogenic. The MV (Type 2) virus was selected as a prototype of a neurotropic strain, while the Mahoney (Type 1) represented a pantropic strain of particular interest since it accounted for almost all of the Cutter-associated human poliomyelitis cases.

In order to differentiate between the neural and humoral routes a differential block of the neural route was produced. Earlier experiments in this laboratory (2) had shown that dry ice freeze of a peripheral nerve prevented the passage of virus which otherwise spread to the CNS following virus dip of the cut nerve end. The same technique was employed in the present work.

Production of experimental poliomyelitis by intramuscular virus injection has been previously reported by a number of investigators. Wenner, who was interested in the distribution of virus in body tissues during the preparalytic phase of infection, used this route because it regularly produced a widely disseminated infection from a localized, readily defined portal of entry (4, 5). In studies of the role of viremia in experimental poliomyelitis, Bodian contrasted intramuscular inoculation with other peripheral and central injection sites (3, 6). In subsequent investigation of the susceptibility of monkeys to poliovirus, Bodian showed that paralytic attack rates following intraneural inoculation were enhanced by concomitant intramuscular virus injection (7).

Intramuscular injection has also been used in studies of the immune mechanism in poliomyelitis. Bodian, in his investigation of the protective effect of passive immunization, found intramuscular injection a useful peripheral route of inoculation, since it resulted in substantial paralytic rates with most of the virus strains tested (8). Morgan, Howe, and Bodian, and later Wenner, found that excellent immune responses were produced by intramuscular injection of active virulent polioviruses (9 to 12).

METHODS

Animals

Cynomolgus monkeys (*Macaca irus*) from the island of Cebu, Philippines, were used throughout. Animals were immature, ranging in weight from 1.5 to 3.0 kilos, and about two-thirds were males. An attempt was made to distribute animals equally by sex and weight, in the various experimental groups. These characteristics did not appear to influence the frequency or site of paralysis, incubation period, viremia, or response to gamma globulin. Most animals were in excellent condition. Of 77 animals, 5 were noted to have lesions (cuts around face—2, infections of face—2, cuts of hand and foot—1), but there was no correlation between site of lesion and initial site of paralysis. All monkeys used had neutralizing antibody titers less than 1:4 for the 3 poliovirus types.

Viruses

Two virus strains were used, the Mahoney (Type 1), and the MV (Type 2). *Mahoney* virus was originally isolated by T. Francis, Jr., from the stool of a human poliomyelitis case occurring in Cleveland, Ohio, in 1941. All experiments were performed with a single Mahoney pool, TC3A, derived from the original source by 11 rhesus intracerebral passages, followed by 3 monkey testicular tissue culture passages. This pool was made in 1952, and has been stored at about −30°C. since that time. The titer of aliquots used in different experiments (tested simultaneously) was $10^{4.5}$ to $10^{5.15}$ TCID 50 per ml. This strain is known to be highly invasive, and capable of producing a marked viremia (6). *MV* virus originated from CNS tissue pooled from fatal human cases at the Rockefeller Institute, New York, 1909 to 1914, and was subsequently passaged many times intracerebrally in rhesus monkeys. Pool IV D587 was made in 1951 as a 10 per cent water suspension of cervical and lumbar cord enlargements of paralyzed rhesus monkeys. Pool V 58-2 was made in 1958 as a 10 per cent water suspension of cervical and lumbar cord enlargements from 9 paralyzed cynomolgus monkeys. Both pools have been stored at −30°C. The MV virus has been used frequently in the study of experimental poliomyelitis, and is known to be highly neurotropic and unadapted to tissue culture.

Viruses were injected into the left calf as a 1 ml. inoculum, with the animal held securely by an assistant. A 20-gauge needle was inserted about 1 cm. into the muscles, taking care to avoid intravascular injection or regurgitation through the needle track.

Nerve Block

All blocks were accomplished by CO_2 freeze of the left sciatic nerve in the sciatic notch (2). A 3 cm. incision was made medial to the greater trochanter, the gluteus maximus retracted laterally, and the sciatic and flexores femoris nerves lifted out of their

bed with a hook. With a wedge of dry ice the nerves were frozen solid to the hook, permitted to melt loose, and refrozen solid. The skin was closed with clips, no hemostasis being required. Associated with self-removal of clips, the incisions became superficially infected in about half the operated animals, and these were treated by application of sulfathiazole powder. Incisions were well healed in 5 to 10 days.

Paralysis

After virus injection monkeys were kept in large cages, which facilitated early detection of paralysis. Animals were checked daily until paralysis began to occur, and then at approximately 12-hour intervals until new cases no longer appeared. Paralyzed animals were carefully examined to determine initial sites of involvement. With the virus strains used paralyzed animals usually progressed to total flaccid quadriplegia, respiratory involvement, prostration, and death. A regular pattern of progression of limb paralysis was noted, in that the interval between onset of paralysis and total flaccidity was quite constant for all limbs in any animal (this interval was 1 to 2 days for the MV virus and rarely over 1 day for the Mahoney virus). Thus, initially paralyzed limbs were always weaker than limbs involved later, and were the first to show total flaccidity. For this reason, if paralysis when first noted involved several extremities, the weakest was considered to have been the site of first paralysis.

RESULTS

MV Virus

The MV virus was used as a 1 ml. inoculation of 10 per cent cord suspension, which probably represents a titer of at least 10^5 PD50, according to previous work in this laboratory (8). This large inoculum accounts for the high paralytic rate (25 of 26 animals) and short incubation period (4 to 5 days). As shown in Table I, initial paralysis was noted in the injected left leg in 21 instances, while 3 animals had bilateral leg involvement, and only one had onset in a distant site (bulbar paralysis).

Neural block by dry ice freeze of the left sciatic was performed just prior to virus injection in 11 animals. This group showed no frank paralysis, from which it appeared that the MV strain spread to the CNS by the neural route, and was unable to utilize other potential routes. Further, the total protection afforded by nerve freeze implied that this procedure provided an absolute block of the neural route. Since the MV virus could not be grown in MK tissue culture, no attempt was made to rule out viremia by exhaustive testing of serum specimens.

Data on antibody responses to MV challenge are limited to animals protected by neural block (experiment 58–1B), since most affected animals either were sacrificed early for cord virus or succumbed to prostrating paralysis. Among 11 animals bled 3 to 7 weeks after virus injection, antibody titers ranged from 1:8 to 1:1000, with a geometric mean of 1:100. It is interesting to speculate whether these considerable titers could be due to the antigenic mass as injected, or whether they imply localized virus

TABLE I

*Effect of Nerve Freeze on Intramuscular Injection of MV Virus**
Frequency and Localization of Paralysis

Expt. No.	Paralytic Rate	Incubation to Paralysis	Site of Initial Paralysis		
			Left Leg	Legs†	Bulbar
Left calf virus injection					
58-1A 58-4A,B,C	25/26	3 days	5		
		5 "	8		1
		5 "	3		
		6 "	4	2	
		7 "	1		
		8 "		1	
		mean = 4.6 days			
Freeze left sciatic nerve followed by left calf virus injection					
58-1B	0/11				

* Expt. 58-1A: pool IV 587, expt. 58-4: pool V 58-2. Inoculum in both experiments: 1 ml. of a 10% cord suspension.

† Paralysis may have had onset in a single leg but had progressed to bilateral flaccidity when first noted.

multiplication with marked increase of available antigen. It is clear that some polioviruses can multiply locally within injected muscle (4, 5). Local multiplication would result in an increase of available antigenic mass and would be reflected in a higher antibody response (10, 13). Antibody responses to a single injection of non-multiplying virus (polio vaccine of high potency) fall in the range 8–125 with a geometric mean titer of 32, in both monkeys (17) and humans (15, 18).

Thus, the 100-fold range in titers in experiment 58-1B may be due in part to differences in the degree of intramuscular virus multiplication, although biological variation in response to injected antigen could, by itself, explain the differences in antibody levels.

Paralysis Following Mahoney Virus

Left calf injection of Mahoney virus, at levels of $10^{3.5}$ or $10^{4.5}$ TCID50, produced a high paralytic rate (19 of 20 animals) in animals without pre-antibody to any poliovirus type. A much lower rate (2 of 11) occurred in a group susceptible to Type 1 but with active antibody to Type 2 virus. This result will be discussed in relation to the viremia studies presented in the next paper.

Tables II and III show paralytic sites following intramuscular virus injection. A comparison is made with intracardiac injection of Mahoney virus based on data previously published from this laboratory (3). Follow-

TABLE II

Intracardiac and Intramuscular Injection of Mahoney Virus
Frequency and Localization of Initial Paralysis

Expt. No.	Paralytic Rate	Limbs Only			Face Only	Face and		
		Arm(s)	Leg(s)	Arm(s) & Leg(s)		Arm(s)	Leg(s)	Arm(s) & Leg(s)
Intracardiac virus injection								
Bodian 1954†	77/158	28	14	10	11	11	2	1
Left calf virus injection*								
58-2	2/11‡							
58-3A	9/9	8	6	1	5		1	
59-1A	10/10							
Freeze left sciatic nerve followed by left calf virus injection								
58-3B	9/10	5	5		7	1		
59-1B	9/10							

* 1 ml. of pool TC3A, either undiluted (expt. 58-3) or 10^{-1} (expts. 58-2, 59-1).
† Retabulation of data from Bodian (ref. 3).
‡ These animals had an active Type 2 immunity at time of Mahoney (Type 1) challenge.

TABLE III

Intracardiac and Intramuscular Injection of Mahoney Virus
Comparison of Sites of Initial Paralysis

Expt. No.	$\frac{\text{Arm(s)}}{\text{Leg(s)}}$†	$\frac{\text{Left Leg}}{\text{Right Leg}}$	$\frac{\text{Left arm}}{\text{Right arm}}$	$\frac{\text{Bulbar}}{\text{All paralytic}}$
Intracardiac virus injection				
Bodian, 1954*	39/16	3/6	17/14	25/77
Left calf virus injection				
58-2				
58-3A	8/7	4/2	5/2	6/21
59-1A				
Freeze left sciatic nerve, followed by left calf virus injection				
58-3B	6/5	5/0	2/1	8/18
59-1B				

* See footnote to Table II.
† Includes animals with arm paralysis only, or leg paralysis only, while those with both arm and leg involvement are excluded.

ing intracardiac injection, which may be considered a non-localizing portal, paralytic rates were high (no direct comparison can be made with intramuscular injection, since the virus inoculum was different). Arm paralysis was twice as frequent as leg paralysis, opposite arms were involved at equal rates, and right was more common than left leg paralysis. After left calf injection the pattern of paralysis was somewhat different, although the numbers are too small to make statistically significant statements. Leg paralysis was more frequent and equal to arm paralysis. There was a suggestion of increased involvement of the left leg (4/2) and left arm (5/2).

It was postulated that if the suggestive increase of left leg paralysis was due to neural spread, then nerve block would obliterate this difference. On the other hand, this procedure would tend to enhance a provoking effect, if operative. Following nerve block there was no reduction in the high paralytic rate (18 of 20), in contrast to the MV virus, where this procedure conferred protection. Leg paralysis was equal to arm paralysis, and the tendency for left leg predominance was more definite (5/0). Throughout these experiments bulbar (usually facial) involvement occurred in about one-third of paralyzed animals, a high rate which was also found after intracardiac injection, and is characteristic of the Mahoney strain.

The relationship between incubation period to paralysis and site of paralysis or size of inoculum is shown in Table IV. The mean incubation period was one day less with undiluted virus than it was with an inoculum of one-tenth that size. Also there was a tendency toward correlation of incubation period and paralytic site in the order: bulbar (6 days), arm (7 days),

TABLE IV

Incubation Period to Paralysis Following Intramuscular Injection of Mahoney Virus

Site(s) of Initial Paralysis	10^0 Virus Expt. 58-3A		10^{-1} Virus Expt. 59-1A		Mean Incubation Period
	Control	Nerve Block*	Control	Nerve Block	
Bulbar	5†, 6	5‡, 5, 5, 6, 6	7, 7, 9, 11	7, 8, 8	6.8 days
Arm(s)	4†, 6, 6, 7, 8, 13	5, 7, 9	7	5, 7, 8	7.1 days
Leg(s)	4, 5, 14	8, 11	5, 5, 7, 10, 12	8, 9, 10	8.3 days
Mean incubation period	7.1 days	6.7 days	8.0 days	7.8 days	7.4 days
	6.9 days		7.9 days		

* Freeze left sciatic nerve followed by left calf virus injection.

† Also tabulated under leg(s).

‡ Also tabulated under arm(s).

leg (8 days). However, it should be noted that the range of incubation periods in all animals (4 to 14 days) was so extensive that there is a great overlap among these groups. This suggests that inoculum and paralytic site play only a partial role in the determination of incubation period.

DISCUSSION

Route of Virus Spread from Periphery to CNS

From these experiments it is clear that poliovirus may spread from an intramuscular injection site to the CNS either along nerves or through the circulation. The relative importance of the two routes is markedly different for different virus strains, but it appears likely that this is a relative rather than an absolute dichotomy. Thus, the neurotropic MV strain caused initial bulbar paralysis in one of 25 monkeys, suggesting possible humoral spread, although intraneural "skipping" could also account for this finding. On the other hand, the pantropic Mahoney spreads readily along neural pathways in the CNS (6), and probably can spread along peripheral nerves. Wenner (5) has shown that the Brunhilde, another pantropic strain, can be isolated from innervating nerve after intramuscular injection and suggests that this is an important route to the CNS.

There is still considerable controversy over the route by which poliovirus reaches the CNS in natural infection (19 to 21), centering around the relative importance of neural and humoral pathways. It has been difficult to evaluate the neural route experimentally because of the numerous potential nervous paths from the natural portals of entry (oropharynx and intestinal tract) to the CNS. If intramuscular injection is used as a model of spread from periphery to CNS, the present experiments may bear on this question. The Mahoney strain, which gives relatively high paralytic rates following feeding of cynomolgus monkeys (22), spreads readily through the circulation to the CNS, when the neural route is blocked. Since viremia also occurs after feeding of Mahoney virus (23), it is clear that humoral spread, by itself, could adequately account for CNS invasion after natural infection with the strain. These data do not totally exclude neural spread as a potential invasive route. However, the results emphasize that a virus capable of causing natural infection must be able to invade extraneural as well as neural tissues, since the neurotropic MV strain which is highly paralytogenic after intramuscular injection, fails to paralyze when fed to monkeys (24, 25).

Intramuscular virus injection may have practical application in testing the invasive properties of the attenuated poliovirus strains developed for human immunization. Most safety testing of attenuated viruses has utilized direct injection into the CNS, and a number of strains have caused paralysis

by this portal (26, 27). However, it has been questioned whether viruses paralytogenic by this route are able to invade the CNS from a peripheral site (28). Recently, intramuscular injection has been used for testing, since it permits the deposit of an exact inoculum into a localized area, and a subsequent study may readily be made of the ability of the virus to invade the CNS by neural and humoral routes (29, 30).

Localization of Paralysis after Intramuscular Virus Injection

These experiments show that neural spread from an intramuscular injection site to the spinal cord is almost invariably associated with localization of initial paralysis in the inoculated extremity. Localization is much less frequent following humoral spread to the CNS. After Mahoney virus injection only 9 of 39 animals (23 per cent) showed localization, which occurred in 71 per cent of 65 paralyzed human cases. This discrepancy may be due to several factors. In the present experiments virus was suspended in BSS, while in the human cases residual virus was present in formalinized vaccine. Bodian (7) found that vaccine apparently was a much more active provoking agent, since an inoculum which when suspended in saline paralyzed 38 per cent of animals, brought down 80 per cent when suspended in vaccine.

An additional factor which probably contributes to the low frequency of localized paralysis following Mahoney virus injection is the large inoculum and consequent high paralytic rate (36/39). A retabulation of Bodian's earlier work on the provoking effect (3) suggests that the frequency of localized paralysis was greater in experiments where the control rate among unprovoked animals was relatively low (Table V). Additional relevant data appear in the next paper which shows that among nerve-blocked animals in which the rise of viremia was delayed there was a high frequency (6/13) of left leg paralysis. This suggests that the provoking effect was playing a more important role when viremia first appeared at low levels, while it was less important when an overwhelming viremia was present soon after injection. Thus the provoking effect may have played a quantitatively more important role among Cutter vaccinees, where less than one case occurred per 1,000 triple-negative children. No attempt was made to test this hypothesis experimentally because of the large number of animals required.

From these considerations it is possible to construct an impression of the interrelationships of viremia, blood-brain barrier, and paralysis. The blood-brain barrier provides a relatively effective defense against virus invasion of the CNS. Presumably small, reparable breaks in this barrier are constantly occurring, so that in the event of a sustained, massive viremia invasion will almost inevitably occur. However, if small

TABLE V

Extent of Provoking Effect According to Paralytic Attack Rate Intracardiac Inoculation of Mahoney Virus with Provoking Injection in Right Calf

(Retabulation of data from Bodian, ref. 3)

Group	Frequency of	Control Rate Below 50%		Control Rate About 50%	
		Rate	Per Cent	Rate	Per Cent
Control	Paralysis	17/59	29%	43/80	54%
Provoked	Paralysis	32/67	48%	80/92	87%
	Observed right leg paralysis	17/32	53%	20/80	25%
	Expected right leg paralysis*			44/80	

* Expected frequency among animals with control rates about 50 per cent is based on animals with control rates below 50 per cent. Chi-square test: P less than .01.

amounts of virus circulate for short periods, the probability is greatly diminished that virus will reach and penetrate a break in the barrier. Under these conditions any event which causes an important break in the barrier will markedly increase the chances of virus penetration. Among such events may be provoking injections, peripheral trauma, violent exercise, pregnancy, and perhaps remote tonsillectomy. Investigation of the mechanism by which breakdown of the blood-brain barrier is produced remains one of the most important gaps in our understanding of the pathogenesis of poliomyelitis.

SUMMARY

A study has been made of poliomyelitis following intramuscular virus injection in the cynomolgus macaque. Interest in this problem was stimulated by the occurrence in the spring of 1955 of 65 cases of human poliomyelitis due to residual virulent virus in a few lots of poliomyelitis vaccine. These cases were notable for the high frequency of paralysis in the inoculated extremity.

1. Following intramuscular injection the highly neurotropic MV strain appeared to spread to the spinal cord by way of peripheral nerve since it was completely blocked by dry ice freeze of the nerve innervating the injected muscle. With this strain paralysis showed a very high frequency of localization in the injected extremity.

2. By contrast, the pantropic Mahoney strain apparently spread by the humoral route, since nerve block afforded no protection. With this strain

there was some evidence of localization in the injected extremity, presumably due to the provoking effect.

3. The Mahoney strain was also responsible for the human cases of inoculation poliomyelitis occurring in 1955. Among these human cases localization was much more frequent than in the present experiments. Evidence is presented which suggests that this difference may be due to an increase in the influence of the provoking effect when the paralytic attack rate is low.

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EXPERIMENTAL POLIOMYELITIS FOLLOWING INTRAMUSCULAR VIRUS INJECTION

II. Viremia and the Effect of Antibody[1, 2]

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Viremia has been firmly established as an important phase in poliovirus infection, both in man (1 to 9) and experimental primates (10 to 19). Viremia can follow introduction of virus by feeding (10, 11, 14, 15, 18), intramuscular (16, 17), or intravascular injection (12, 13). The titer and persistence of virus in the blood is influenced by the strain utilized (12), the size of the inoculum (16, 17), and the presence of homologous antibody (18).

In spite of this sizable body of data there is still considerable controversy regarding the role of viremia in the spread of virus to the central nervous system after infection via the natural oral portal of entry (20 to 23). Specifically, the practical difficulty of blocking all potential neural routes from gut to CNS have made it impossible to determine the frequency of CNS invasion when the viremia route alone is available. In the course of the present study of poliomyelitis following intramuscular virus injection (see the preceding paper), it became apparent that this objection could be overcome. Because of the localized portal it was possible to block the neural route effectively, as shown by the complete protection conferred against a neurotropic strain by nerve freeze. However, in spite of neural block the pantropic Mahoney strain continued to cause paralysis at a very high rate.

Thus it appeared that these experimental conditions created a situation where viremia was playing a vital and unambiguous role in the passage of virus to the CNS. For this reason it was particularly interesting to follow the course of viremia in the animals used in this study. Because frequent bleedings had been obtained it was possible to explore the parameters of viremia in considerable detail and to relate them to the overt manifestations of infection.

The data also provided considerable information on the interrelationships of viremia and antibody response. In one experiment the animals used had an active immunity to a heterologous virus type and the re-

[1] Aided by a grant from the National Foundation, Inc.

[2] We wish to thank Mr. William D. McClaskey for expert technical assistance.

TABLE I

Summary of Experiments

Left Calf Injection of 1 ml. Type 1 Mahoney Virus, Pool TC3A

Expt. No.	Pre-existing Antibody	Concentration of Virus Inoculum	Left Sciatic Nerve Freeze	Bleedings	Animals	
					No.	Paralyzed
58-2	Type 2	10^{-1}		Daily on 6/11	11	2
58-3A	None	10^{0}		Daily	9	9
B	None	10^{0}	Day of infection	Daily	10	9
59-1A	None	10^{-1}		Alternate days	10	10
B	None	10^{-1}	Day of infection	Alternate days	9	9

sults suggested a possible mechanism for the phenomenon of heterologous protection which has been reported for poliomyelitis (24 to 30).

METHODS

Animals, viruses, and most procedures are described in the preceding paper. Of the 2 virus strains used, the MV (Type 2) could not be grown in monkey kidney tissue culture. This report is therefore concerned solely with experiments in which Type 1 Mahoney virus was injected into the left calf of cynomolgus monkeys (*Macaca irus* group). Protocols of these experiments are summarized in Table I.

Bleeding

Blood was obtained by cardiac puncture (12). Deaths due to pericardial hemorrhage and tamponade occurred 2 times in over 400 bleedings of 77 animals. Clotted blood was spun at 600 rpm for 20 minutes, serum decanted, and stored at −30°C.

Tissue Culture

Tests were performed with cynomolgus monkey kidney cells. Animals were bled out under ether anesthesia, kidneys removed, cut into 2 to 3 mm. fragments, and digested in 60 to 90 ml. of 0.25 gm. per cent bactotrypsin (Difco Labs.) in Hank's BSS, at room temperature with constant magnetic stirring. At hourly intervals suspended cells were poured off and a fresh aliquot of trypsin added. Digests of the first 2 hours were discarded, and cells collected from 2 to 4.5 hours were saved, washed twice in Hank's BSS, and suspended in medium 59 (31).

Medium 59 is Hank's or Earle's BSS to 1 liter of which is added: lactalbumin hydrolysate (Balto. Biol. Co.), 5 gm.; phenol red, 0.04 gm.; and 2.5 ml. 1 N NaOH. After sterilization by filtration through a Seitz EK pad, No. 6, the following are added as sterile solutions: 30 per cent bovine albumin (Armour), 4 ml.; streptomycin 0.1 gm.; and crystalline penicillin G, 100,000 U. The pH of the complete medium is 7.5.

Virus Isolation

Serum specimens were screened and titered in rubber-stoppered 75 × 12 mm. glass tubes, simultaneously inoculated with 0.5 ml. of diluted specimen and 0.25 ml. of medium 59 (Hank's), containing 125,000 cells, by hemocytometer count. Tubes were incubated for 7 to 10 days at 37°C. and read by color and microscopic changes. Negative speci-

mens were further tested at least once in two to four 150 × 16 mm. screw-cap glass tubes. These tubes were set up with 500,000 MK cells in 3 ml. of medium 59 (Hank's or Earle's) with 2 per cent calf serum (Microbiological Associates). Medium 59 was changed on day 4 and a satisfactory monolayer of cells was present in 50 to 95 per cent of tubes by day 6 or 7. Medium 59 was again changed (without calf serum) and 0.1 to 0.25 ml. of specimen inoculated, with further changes on days 2 to 3 and 5 to 6 after inoculation. Tubes were examined microscopically every 1 to 2 days for 7 to 10 days after inoculation. Cytopathic changes due to poliovirus rarely appeared before day 2 or after day 6 of inoculation.

All titers are expressed as the concentration of virus per ml. serum, or the reciprocal of the highest dilution of serum which infected 50 per cent of tubes, calculated according to Reed and Muench (32). Virus typings were performed on representative viremic sera by the addition of an equal volume of a 50-fold dilution of standard lyophilized monkey antiserum[3] to serial dilutions of serum, followed by incubation at room temperature for 1 to 3 hours before addition of 125,000 MK cells. Positive sera yielded Type 1 poliovirus only.

Neutralizing Antibodies

Sera were titered for antibodies in 75 × 12 mm. tubes. Connaught standard viruses[4], 35 to 750 ID50 in 0.25 ml. of medium 59 (Hank's), were incubated with 0.25 ml. of each serum dilution for 1 to 3 hours at room temperature, 125,000 MK cells added, incubated at 37°C., and read at 7 to 10 days. Antibody titers are expressed as the reciprocal of the highest dilution of 0.25 ml. serum which neutralized the standard virus.

Repeat titrations were influenced by the amount of virus used in different tissue culture runs, and also showed random variation. The data suggested that, in the range 35 to 670 ID50 of virus, a 4-fold increase in virus caused a 2-fold decrease in estimated titer, a relationship quantitatively similar to that reported by Wenner (33). For this reason sera from each experiment were titered in a single TC run. Random variations between 2 determinations on one specimen averaged 1.7-fold, and for 95 per cent of sera (2 S.D.s) were 3-fold or less. In all experiments neutralizing antibody responses were homotypic for the virus type injected.

RESULTS

The Course of Viremia

All animals without pre-antibody (experiments 58-3 and 59-1) showed a marked viremia with Mahoney virus. The daily course of viremia appears in Table II and Figure 1, which shows an influence of the size of virus inoculum at the two levels used (dilutions of 10^0 and 10^{-1}). With the larger inoculum, viremia appeared rapidly and was present in almost all animals on days 1, 2, and 3 after injection, and then fell off rapidly so that all animals were negative on day 6. With the smaller inoculum, viremia appeared more gradually, was present in almost all animals on

[3] Prepared by H. A. Wenner (33), and made available by the National Foundation, Inc.

[4] Prepared by the Connaught Laboratories (33), and made available by the National Foundation, Inc.

TABLE II

*Course of Viremia Following Intramuscular Injection of Mahoney Virus**

Days after Virus Injection	Proportion of Sera Showing Viremia			G.M.T. per ml. Serum		
	10^0 virus	10^{-1} virus		10^0 virus	10^{-1} virus	
	58-3	59-1	58-2	58-3	59-1	58-2
1	17/19	5/10	1/6	10	8	2
2	18/19	5/10	0/6	200	16	
3	18/19	10/10	2/6	200	1,000	2
4	13/19	9/10	2/6	50	250	2
5	5/10	6/10	2/6	20	16	2
6	0/9	5/8	0/6		250	
7		1/5			1	
8		2/6			2	
9		1/8			1	
Frequency of viremia	19/19	19/19	4/6			

* Expt. 58-2: these animals had Type 2 antibody at the time of Mahoney (Type 1) challenge. Expt. 59-1: animals bled on alternate days. Geometric mean titers (G.M.T.) include negative sera. Frequency of viremia: the number of animals showing viremia on one or more occasions.

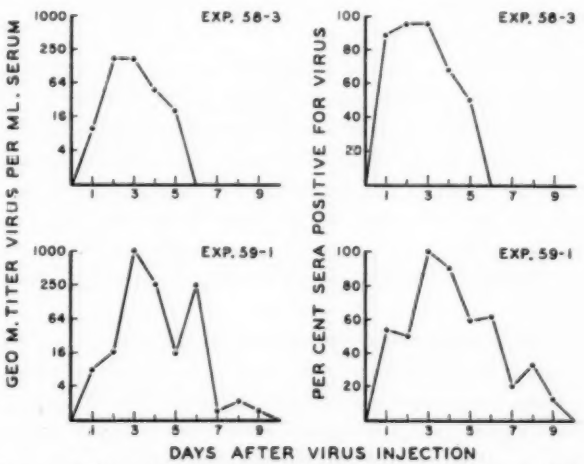

FIG. 1. Course of viremia following intramuscular injection of Mahoney virus.

days 3 and 4, and waned slowly, not disappearing until day 10. The geometric mean titers for the two groups show a similar picture.

These differences are further documented in Table III. Onset of viremia was one day later with the smaller inoculum. Once viremia ap-

TABLE III

*Onset, Duration, and Peak of Viremia**

		Expt. 58-3 10^{0} virus	Expt. 59-1 10^{-1} Virus
No. of animals		19	19
Days from injection to onset of viremia	Range	0–2	0–5
	Mean	.5 days	1.3 days
Days from onset to peak of viremia	Range	0–4	0–3
	Mean	2.1 days	2.2 days
Duration of viremia in days	Range	1–6	3–8
	Mean	4.5 days	5.0 days
Peak viremia titer per ml. serum	Range	10–16,000	500–250,000
	Mean	1,500	5,000

* Bleedings in Expt. 59-1 were taken on alternate days and data for days not available were estimated by interpolation prior to making the tabulations in this and succeeding tables.

peared, the time to reach peak titer (2 days) and the total duration (5 days) were similar in both groups. Peak titers tended to be slightly higher with the smaller (5,000 TCID50 per ml. serum) than with the larger inoculum (1,500 TCID50).

The course of viremia varied somewhat among animals receiving the same virus inoculum, as appears in Figure 2. In this figure and the remaining tables, data for experiment 59-1 were modified by interpolation of estimated viremia titers, since bleedings were made every other day. Animals given 10^{-1} virus fall into three groups, with viremia peaking on days 2, 3 to 4, and 5 to 6, respectively. After 10^{0} virus two groups could be distinguished, with peaks on days 2 and 3 to 4. Thus there was considerable overlap in the course of viremias following the larger and smaller inoculum.

Looking at the combined results of both experiments, individual variations in the parameters of viremia may be summarized: injection to onset, 1 to 5 days; onset to peak, 0 to 4 days; duration, 1 to 8 days; peak titer, 10 to 250,000 TCID50. Because of this variation it was of interest to look for relationships between the parameters of viremia. Cross-tabulations showed in one of two experiments a tendency for association of higher peak titers with a longer interval from onset of viremia to peak, and in both experiments an association of greater viremia duration with a longer interval to peak titer. The lack of other associations may be due in part to the small number of animals and to the limited range of virus inocula.

The parameters of viremia are tabulated against clinical characteristics in Tables IV and V. Incubation periods tend to be longer in animals where

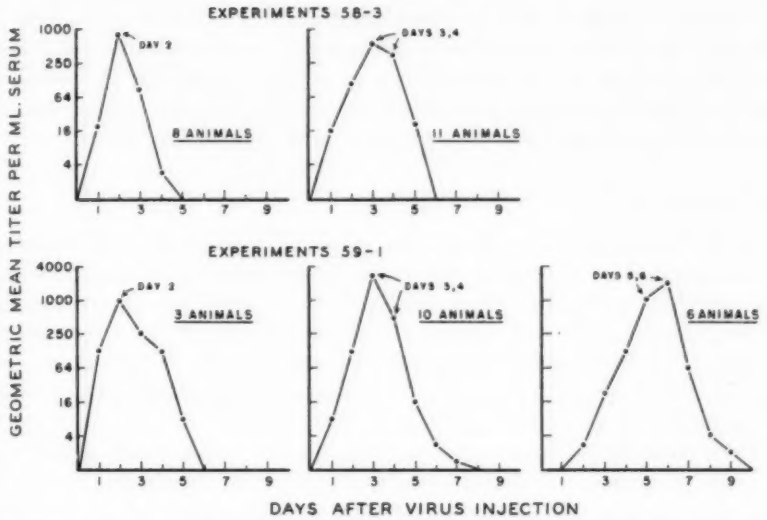

FIG. 2. Variation in the course of viremia, following intramuscular injection of Mahoney virus.

TABLE IV

*Onset of Viremia and Incubation Period**

Virus Injection to Onset of Viremia	No. of Animals	Incubation to Paralysis	
		Range	Mean
1 day	25	4–13	6.9 days
2 days	7	7–14	
3 days	4	8–9	9.1 days
4–5 days	1	10	

* Results of Expts. 58-3 and 59-1 were similar and are combined in this table.

TABLE V

*Course of Viremia and Site of First Paralysis**

Virus Injection to Viremia 100 TCID50 per ml. Serum	No. of Animals	Site of First Paralysis			
		Bulbar	Arm(s)	Leg(s)	Left Leg
1–2 days	24	6	12	6	2
3–5 days	13	6	1	6	6

* Combined results of Expts. 58-3 and 59-1.

the interval between virus injection and appearance of viremia is greater. Although this is not an entirely consistent trend, the similarity between the number of days by which viremia is delayed and the number of days increase in incubation period suggests a real relationship.

Turning to sites of first paralysis, animals in which the appearance of viremia was delayed showed a low frequency of arm paralysis and a high frequency of left leg paralysis (6/13) compared to animals in which viremia appeared early (2/24 with left leg paralysis). This finding was discussed in the previous paper in relation to the provoking effect.

Homologous Antibody Response

Serial bleedings, for viremia also provided considerable information on the early phase of neutralizing antibody development. Table VI shows that antibody appeared 3 to 9 days after virus injection, about one day earlier following 10^0 virus (median: 5 days) than for 10^{-1} virus (median: 6 days). Antibody appeared earlier among animals in which viremia peaked earlier. Delay in the peak of viremia was correlated with a somewhat greater delay in the appearance of antibody.

The relationship between the disappearance of viremia and appearance of antibody is shown in Table VII. On the day prior to the appearance of antibody, viremia was present in all of 26 animals tested. On the first day of antibody, viremia was present in 4 of 35 animals at low titers (8 to 32 TCID50), and never thereafter. On the day before antibody viremia titers were definitely on the wane among animals receiving 10^{-1} virus, but were still high among animals receiving 10^0 virus. The geometric mean antibody titer was low (1:4) on the day of appearance and rose at an approximately logarithmic rate to about 1:64 one week later.

There was no relationship between the time of antibody appearance and incubation to paralysis. Antibody appeared between 11 days before

TABLE VI

*Time of Peak Viremia Titer and of Appearance of Antibody**

Virus Injection to Appearance of Antibody (days)	Virus Injection to Peak Viremia Titer	
	No. of Animals	Mean
3	1	2.1 days
4	5	
5	11	2.9 days
6	11	3.5 days
7	1	
8	5	5.2 days
9	1	

* Combined results of Experiments 58-3 and 59-1. Excludes 3 animals paralyzed before antibody appeared.

TABLE VII

Disappearance of Viremia in Relation to Appearance of Antibody

Day of Antibody	Geometric Mean Viremia per ml. Serum		Geometric Mean Antibody Titer*	
	Expt. 58-3 10^{0} Virus	Expt. 59-1 10^{-1} Virus	Expt. 58-3 10^{0} Virus	Expt. 59-1 10^{-1} Virus
−6	16	2		
−5	20	16		
−4	240	125		
−3	140	750		
−2	170	2,000		
−1	400	50	—	—
1	—	2	4	4
2			8	8
3			8	8
4			16	8
5			32	12
6			64	16
7			64	50

* Titers include negative sera, and are expressed as the highest dilution which neutralizes about 100 TCID50 of standard virus.

to one day after the onset of paralysis. Similar findings were reported by Wenner (15).

Effect of Pre-existing Heterotypic Antibody

Antibody titers were of particular interest in experiment 58-2 because the inoculum (10^{-1} virus) produced a much lower paralytic rate (2/11) than it did in experiment 59-1 (18/20). Moreover, of 6 animals followed daily in experiment 58-2 only 4 showed viremia, and among these virus appeared sporadically and in very low titer. This was in marked contrast to experiment 59-1 where each of 19 animals developed viremia and titers were high, as Table II shows. It appears likely that the low paralytic rate seen in experiment 58-2 was due to minimal, transient nature of the viremia seen in these animals, as suggested by Bodian (12).

Since experiment 58-2 was the only one in which the animals had antibody (Type 2) at the time of Mahoney (Type 1) challenge, the findings suggest partial protection from a previously acquired active immunity to a heterologous virus. Table VIII shows the viremia and antibody pattern in the 6 animals bled daily. At the time of Type 1 challenge, Type 2 antibodies ranged from 1:32 to 1:1,000. From experiment 59-1 the appearance of Type 1 antibody would first be expected 5 to 9 days after challenge. In fact, Type 1 antibody was present in 4 of 6 animals and 6 of 19 bleedings obtained on days 1 through 4.

It is possible that this early antibody was formed in response to the

TABLE VIII

*Type 1 Viremia and Antibody Response in the Presence of Active Type 2 Immunity**

Animal No.	Type 2 Antibody Titer	Type 1 Response	Days after Injection of Type 1 Virus												
			0	1	2	3	4	5	6	7	8	9	10	11	12	5 wks.
12	125	*V*				*20*	*40*									
		Ab	<4		<2			<2	4	16	16	64	64	125	>250	1,000
14	1,000	*V*														
		Ab	<4	<2	<2	<2	<2	4	4	<2	<2	4	<2	4	<2	2
16	32	*V*	*10*			*10*		*10*								
		Ab	<4	<2	<2	<2	2	2	4	4	32	64	16	125	>250	500
18	250	*V*														
		Ab	<4	4	<2	<2	2	<2	2	2	4	8	16	32	32	32
20	125	*V*					*10*									
		Ab	<4		<2	2		4	16	64	64		64	64	125	500
22	32	*V*						*10*								
		Ab	<4	4	2	<2	<2	8		4	8	8	8	32	16	16

* Results of Expt. 58-2. *V*: Viremia titer as TCID50 per ml. serum. All sera were tested 2 to 5 times, but only positive results are indicated. Ab: Type 1 antibody as reciprocal of serum dilution. Type 2 antibody titers refer to day 1. Among the 11 animals in experiment 58-2 two cases of frank paralysis developed, both in the group of 5 animals not subjected to serial bleeding.

previous Type 2 exposure and predated the Type 1 challenge, rather than representing a precocious response to this challenge. Although no antibody was detected at the 1:4 level just prior to Mahoney injection, antibody was found in 2 of 4 bloods on the next day, which is probably too early for an accelerated response. Also, antibody levels did not rise more rapidly than in experiment 59-1, since levels of 1:8 or greater were first found 5 to 9 days after challenge (also 5 to 9 days in experiment 59-1) and at 9 days after challenge ranged from 4 to 64 with a geometric mean of 16 (4 to 32 with a mean of 8 in experiment 59-1). Finally, of 6 animals followed for 5 weeks, one never developed a titer above 1:4 and 2 others never rose above 1:32. These limited responses could be explained by low levels of pre-antibody which neutralized much of the challenge virus before it could reach sites of antibody formation.

DISCUSSION

The Course of Viremia, Incubation Period, and Site of Paralysis

The latent period from injection to onset of viremia increases with decreasing size of intramuscular inoculum. Wenner has reported similar

findings, and shown that during the latent period virus is multiplying within the injected muscle and lymph nodes draining the area (16, 17). In groups of animals where onset and rise of viremia are delayed, there tends to be a corresponding delay in the onset of paralysis. The fact that these short delays in appearance of viremia are reflected in the incubation period implies that virus penetrates the CNS relatively quickly after it enters the circulation, since a lag of more than 1 or 2 days would probably damp out the effect of delayed viremia. These findings are in agreement with Bodian's observation (13) that the incubation period was 1 to 2 days longer after intracardiac than after intracerebral inoculation. This difference corresponded to the 1 to 2 day lag between intracardiac inoculation and onset of viremia, again implying that virus invaded the CNS shortly after it entered the circulation.

Thus the incubation period to paralysis may be broken down into three components: multiplication of virus in a peripheral site, onset of viremia to CNS invasion, and incubation within the CNS. For the conditions of the present experiments these components may be estimated to have mean values: peripheral incubation, 1 to 2 days; viremia to CNS invasion, about 1 day; incubation within the CNS, 5 to 6 days. Variation around each mean is roughly proportional to the value of the mean, according to tabulations of these data and of those previously published by Bodian (34). In other words, variation in overall incubation is primarily due to variation in incubation within the CNS. Factors which have an important influence on each component of the incubation period are: virus inoculum, growth characteristics of the virus strain, and the specific peripheral or central locus within which virus is multiplying.

Incubation period varied according to initial site of paralysis, increasing as the paralytic focus moved caudally from medulla to cervical to lumbar cord. A similar finding has been previously reported (12). There was no correlation between the course of viremia and initial site of paralysis, with the special exception of provoked paralysis which was noted above. Thus this observation probably reflects differences in incubation period after virus invasion of different regions of the CNS.

Appearance of Antibody and the Close of Viremia

The present experiments have brought out clearly the role of antibody in the termination of viremia. Serum neutralizing antibody usually made its appearance on the day following the termination of viremia. In a few animals antibody was first noted, at low levels, on the last day of viremia. The detection of virus and antibody in the same serum specimen when both are at low levels suggests the possibility that there is considerable overlap between the shedding of virus and antibody into the circulation,

with a neutralization of antibody by an excess of virus during the height of viremia and the subsequent neutralization of virus by an excess of antibody. However, both components would be detected only on rare occasions when they are present in approximately equal proportion. The application of techniques for the recovery of virus and antigen from a neutralized mixture (35 to 37) might elucidate this point.

These considerations suggest that the severe viremia produced by the Mahoney strain comes to a close when sufficient amounts of circulating antibody appear, and that this may occur prior to the burning out of infection in tissues which shed virus into the circulation. Previously published comparisons of the Mahoney and other virus strains (12) shows that when viremia is minimal it may close spontaneously prior to the appearance of circulating antibody.

Effect of Heterotypic Antibody on Infection

In one group of animals which had a pre-existing active Type 2 immunity, Type 1 challenge failed to cause the usual high rate of paralysis. Evidence that prior heterotypic antibody protects against viremia (38), or against the paralytic consequences of infection in monkeys (24, 25) and in humans (26) has been reported previously. This is just one manifestation of the tendency for Type 2 poliovirus antigens to cross-react with both Types 1 and 3 (26 to 30).

The present data are of interest because they provide some information about the mechanism of heterotypic protection. Heterotypic protection was clearly associated with a marked damping of viremia. This could be due to an accelerated antibody response to the challenge virus, as suggested by Salk (26), or to pre-existing antibody against the challenge virus, formed in response to the previous heterotypic infection, a phenomenon reported by Sabin (27). As noted in the section on results, there was no sign of an accelerated antibody response but considerable indirect evidence that much of the challenge inoculum had been rapidly neutralized by pre-antibody present at very low or undetected levels.

SUMMARY

A study was made of viremia in cynomolgus macaques after intramuscular injection of the pantropic Type 1 Mahoney virus.

1. With the large inocula which were used, Mahoney virus caused a massive viremia. Variations in the parameters of viremia were influenced both by the size of the virus inoculum and by individual variability. Viremia, in turn, was one of the factors which determined incubation period and sites of initial paralysis.

2. In these experiments the development of serum antibody appeared to be responsible for the waning and termination of viremia, although this is not the case when viremia is moderate or minimal.

3. In one experiment pre-existing active immunity to a heterologous virus type conferred partial protection against paralysis, which was probably related to the damping of viremia in this group of animals. There was evidence that the damping of viremia was due to neutralization of much of the challenge virus by pre-antibody present at very low levels, rather than to the occurrence of a precocious antibody response.

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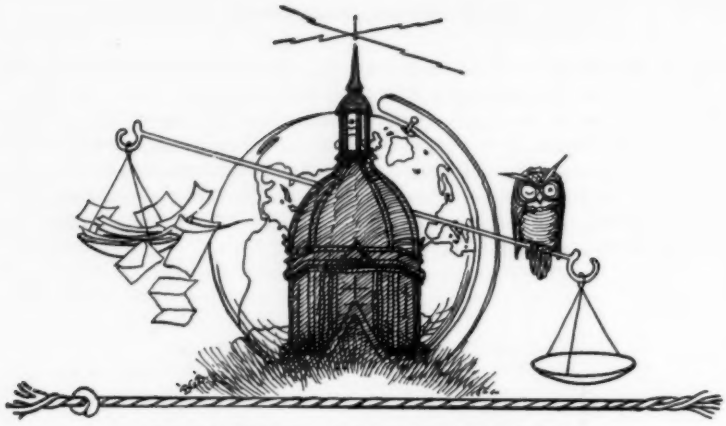

BOOK REVIEWS

Congenital Malformations of the Heart, revised edition. Vol. I, General Considerations. By HELEN B. TAUSSIG. 204 pp., $4.75. Published for The Commonwealth Fund by Harvard University Press, Cambridge, Mass., 1960.

Volume I of this long awaited revision of Dr. Taussig's text is appropriately subtitled "General Considerations". The author has wisely isolated the general principles of embryology, physiology, diagnosis and treatment for consideration in this introductory volume, thus avoiding the repetition so often present in other text books in which basic principles must be discussed anew with each disease. An outstanding example of the material in the first chapter is the section entitled "Differences in The Approach to The Problems of Adults and Infants". In Chapter 2, the author, a strong advocate of cardiac fluoroscopy by the cardiologist, has set forth in the form of diagrams the general principles of cardiac fluoroscopy. Angiocardiography and cardiac catheterization are discussed in the third chapter. The angiocardiograms have been retouched to show margins of chambers, great vessels and valves. The chapter on medical care contains practical detailed information about management, therapeutic procedures and drugs. Patients with congenital heart disease frequently ask about marriage, pregnancy and the risk of congenital malformations in offspring. These difficult questions are frankly discussed. In summary, this volume contains the essence of the author's general wisdom about congenital heart disease and is well worth reading alone, but will be especially useful as background for the second volume dealing with specific malformations.

RICHARD S. ROSS

Congenital Malformations of the Heart, revised edition. Volume II, Specific Malformations. By HELEN B. TAUSSIG. 1049 pp., $17.50. Published for The Commonwealth Fund by Harvard University Press, Cambridge, Mass., 1960.

In this second volume dealing with specific congenital malformations of the heart, the plan of each chapter is similar to that used in the first edition. The circulatory diagrams, anatomical drawings, and radiological silhouettes will be familiar to students of the first edition. The circulatory diagrams are in black and white rather than in color, but their usefulness has not been impaired by this change. Many more electrocardiograms are included in this edition and they are displayed in a simple, standard block form

which makes for easy reference. The anatomical drawings are as before an outstanding feature of the book. The drawings of the heart as it appears in situ give the student an understanding of normal and pathological anatomy which cannot be acquired from photographs of fixed specimens or from circulatory diagrams.

A great deal of basic material has been removed from this volume and placed in Volume I. Even so, Volume II is approximately twice as big as the entire first edition published in 1947. This, of course, reflects the expansion of knowledge which has occurred during the past 13 years. The author has summarized and synthesized opinion on malformations which have been recognized only in the last few years. An example of this is Chapter 31 which deals with Corrected Transposition of the Great Vessels. This volume contains a magnificent 27 page index for its 1007 pages and will take the place of the first edition as the standard reference book on congenital heart disease.

RICHARD S. ROSS

Respiration. Physiologic Principles and Their Clinical Applications. By P. H. ROSSIER, A. A. BÜHLMANN, AND K. WIESINGER. Edited and translated from the German edition by PETER C. LUCHSINGER AND KENNETH M. MOSER. 505 pp., $15.75. The C. V. Mosby Co., St. Louis, Mo., 1960.

The translation into English of the text *Respiration* by Rossier, Bühlmann, and Wiesinger is an important event for anyone interested in human pulmonary physiology and pathophysiology. The text of 404 pages, an appendix of equations and tables, and an excellent 80 page bibliography divided by subject serves a need not met by any other single work. The book is divided into four parts: I Normal physiology of respiration; II Investigative methods in pulmonary function; III Pathophysiology of respiration; IV Pulmonary insufficiency in clinical practice.

The rapid advances in the field in recent years, particularly the newer terminology, have made most of the earlier standard works in respiration useless to the student. In this text historical perspectives are preserved in nearly every section, but at the same time current definitions and symbols are used. There are a number of excellent diagrams and tables. The clinical section is enhanced by the presentation of measurements on individual patients. When interpretations of data are controversial, usually both sides are presented.

The decision of the authors to include a large bibliography divided by subject has some merit for the investigator. It often provides frustration for the reader, who anxious to check on a given statement, may have trouble finding the suitable reference. In some instances papers quoted in the text by author and year of publication are not listed in the bibliography.

In a single volume on physiology and pathophysiology of respiration a number of omissions are understandable. For example there is no mention of the respiratory problems of infants and children. There is some regret in including a section on artificial respiration which is not only inadequate in failing to mention methods dependent on gravity, but misleading in the comments on positive versus negative pressure breathing. Likewise the section on oxygen therapy is so brief as to have been better omitted.

The few defects mentioned above do not detract from the significance of the translation of this important book. The student, clinician, and physiologist alike will find it good reading and a valuable addition to the library.

MARY ELLEN AVERY

Tumors of Childhood. A Clinical Treatise. By HOWARD W. DARGEON. 476 pp., $20.00. Paul B. Hoeber, Inc., New York, N. Y.

During childhood, second only to trauma, cancer in the 1950's was the most frequent cause of death. It seems appropriate that Dr. H. W. Dargeon, Pediatrician at the Memorial Center in New York, has prepared in careful, convenient and comprehensive manner this monograph on tumors in infancy and childhood. This clinical treatise commences with a lucid general discussion of the history, etiology, behavior, diagnosis, prognosis and management of neoplasms. Whenever pertinent, the author emphasizes special features of tumors in infancy and childhood, such as familial occurrence, congenital neoplasms, complications associated with radiotherapy, and psychiatric or psychologic management of patient and family.

The major portion of the text (pages 85 to 454) describes in detail frequency, natural history, diagnosis, therapy and prognosis of specific neoplasms (fetal, teratomas, head and neck, central and peripheral nervous system, thoracic, abdomen and gastro-intestinal tract, genito-urinary tract, bone, hematopoietic sites, angiomas, skin and soft tissues, reticulo-endothelioses and rare tumors). The uniform pattern of the discussions and the completeness of the index greatly facilitate location of specific information. Dr. Dargeon precisely summarizes his experience gained from the careful analysis of more than 6000 cases studied during the past 25 years. In addition, his conclusions are based both on his own experiences as well as on the statistical reports available from the literature. A fine bibliography follows each chapter.

Certain minor details warrant clarification. For instance, on page 400, the discussion of desmoid tumors and juvenile fibromastosis states "the encapsulated lesions grow slowly, and while they do not generally metastasize, they sometimes cause serious complications by their extensive invasion of adjacent structures". Elsewhere (pages 44 and 209) it is stated correctly and concisely that desmoid tumors do not metastasize.

A heavy glossy stock has been used in printing the easily read, large and sharp type. The clinical illustrations, roentgenograms, gross pathologic specimens and tables are of good quality and add to the text. This monograph is warmly recommended to all who are concerned with the treatment of tumors in infants and children.

SUMNER WOOD, JR.

Diseases of the Nervous System in Infancy, Childhood and Adolescence, 4th edition. Completely revised and reset from new type. By FRANK R. FORD. 1548 pp., $29.50. Charles C Thomas, Publisher, Springfield, Ill.

This famous text, now in its fourth edition, retains its status as the definitive and encyclopedic work on the diseases of the developing nervous system. Inevitably, it has grown, the text alone having increased by one hundred ninety-two pages. This increase is not mere accretion, since all sections show clear evidence of revision, while some forty conditions are either newly discussed, or markedly altered in their presentation. The volume is enlivened with several new illustrations, well chosen to delight those interested in clinical pathology. A special pleasure to the reader is a new, detailed, and excellent index, replacing the former one, of a quarter its size.

The book is one of great value and of great charm. Its value lies in its quality of encyclopedic completeness without sacrifice of the quality of individual experience and evaluation. There can be little in the way of real or supposed aberrations of the developing nervous system from congenital chin trembling to leprosy, which is not here in succinct, accurate account, complete with critical references and brightened with a line of personal reminiscence, a generous credit to a colleague, or a brief case

note. The accounts have always that ring of unassuming assurance that can come only with years of seeing patients and syndromes come and go.

This is not to imply that the book is perfect; to do so would be to denigrate it by lack of that considered criticism which marks its pages. The introductory chapter, dealing with the clinical aspects of the nervous system, a small masterpiece in its own right, would no doubt profit from discussion of newer physiological studies, especially on the cerebellum. A carping pathologist might question the labelling of the brain on p. 169 as "atrophic lobar sclerosis," and rebel at the identification of rhabdomyoma with the diffuse glycogenosis of cardiomegalic glycogen storage disease. Most clinicians would question the use of dihydrostreptomycin in CNS tuberculosis. But these are small considerations to set over against the value of such minor classics as the descriptions of acute infantile hemiplegia, congenital indifference to pain, migraine in childhood, epidemic encephalitis lethargica, and the priceless chapter on psychogenic disorders simulating organic disease.

The great charm of the volume rests in its clear expression of its author's personality. As his old friend Sir Francis Walshe has put it, it is all his own work. In this day of multi-authored textbooks, editorially pruned of their smallest sprigs of individuality, it is hard to find a work in which opinions, frankly expressed, have the weight and unity of one man's own enormous clinical experience. The reader may or may not agree with Dr. Ford, but he cannot fail to realize that he measures his opinion against that of a man who brings much thought, and endless observation and study, to the problem. The keen estimates of other men's work, set off by the humor of brief clinical asides—the strongly maternal woman, much-married in vain search of offspring free of cerebral lipidosis, the child whose phosphorus poisoning produced such distressing pyrotechnic effects—bespeak the pungent personality and the keen clinician whose book continues as the indispensable volume for all those who labor with the neurology of childhood.

DAVID CLARK

BOOKS RECEIVED FOR REVIEW

Basic Pharmacology for Nurses, 2nd edition. By JESSIE E. SQUIRE. 275 pp., $3.50. The C. V. Mosby Co., St. Louis, Mo., 1961.

Cardiovascular Dynamics, 2nd edition. By ROBERT F. RUSHMER. 503 pp., $12.50. W. B. Saunders Co., Philadelphia, Pa., 1961.

Currenty Therapy—1961. Edited by HOWARD F. CONN. 806 pp., $12.50. W. B. Saunders Co., Philadelphia, Pa.

Haematology and Blood Groups. Papers from the British Medical Bulletin. Edited by D. A. G. GALTON AND K. L. G. GOLDSMITH. 169 pp., $4.00. The University of Chicago Press, Chicago, Ill., 1961.

New and Nonofficial Drugs, 1961. Evaluated by A.M.A. COUNCIL ON DRUGS. 849 pp., $4.00. J. B. Lippincott Co., Philadelphia, Pa.

Pathology. A Dynamic Introduction to Medicine and Surgery. By THOMAS M. PEERY AND FRANK N. MILLER, JR. 626 pp., $5.50. Little, Brown & Co., Boston, Mass., 1961.

Persuasion and Healing. A Comparative Study of Psychotherapy. By JEROME D. FRANK. 282 pp., $5.50. The Johns Hopkins Press, Baltimore, Md., 1961.

Pharmacology. The Nature, Action and Use of Drugs, 2nd edition. By HARRY BECKMAN. 805 pp., $15.50. W. B. Saunders Co., Philadelphia Pa., 1961.

A Student's Guide to Obstetrics and Gynaecology. By CHRISTOPHER J. DEWHURST. 229 pp., $4.00. J. B. Lippincott Co., Philadelphia, Pa., 1960.

Surgery for Nurses, 7th edition. By JAMES MORONEY. With a Foreword by DOROTHY M. SMITH. 792 pp., $7.50. E & S Livingstone, Edinburgh & London, 1961. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

Surgical Diseases of the Chest. Edited by BRIAN BLADES. 580 pp., $22.00. The C. V. Mosby Co., St. Louis, Mo., 1961.

A Symposium on Light and Life. Edited by WILLIAM D. MCELROY AND BENTLEY GLASS. 924 pp., $15.00. Sponsored by The McCollum-Pratt Institute of the Johns Hopkins University. The Johns Hopkins Press, Baltimore, Md., 1961.

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A Synopsis of Contemporary Psychiatry, 2nd edition. By GEORGE A. ULETT AND D. WELLS GOODRICH. 309 pp., $6.50. The C. V. Mosby Co., St. Louis, Mo., 1961.

Textbook of Medical Physiology, 2nd edition. By ARTHUR C. GUYTON. 1181 pp., $15.50. W. B. Saunders Co., Philadelphia, Pa., 1961.

SIMULTANEOUS CEREBROSPINAL FLUID AND SERUM ACID-BASE BALANCE, IONIC PATTERNS AND IONIC AND OSMOLALITY DISTRIBUTION RATIOS

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The purpose of this communication is to present simultaneous studies of the blood, serum and cerebrospinal fluid of six patients with neurosurgical conditions in terms of the acid-base balance patterns, ionic patterns, osmolality and ionic distribution ratios of these fluids. All will be discussed in relation to the clinical state of the individual patient and compared to studies of controls as well as noting variations in those patients with multiple observations. In studies such as these it is advisable to examine the cerebrospinal fluid and serum simultaneously (1, 2).

The data on these patients will be discussed from three viewpoints: 1) the acid-base balance patterns of the blood and cerebrospinal fluid, 2) the ionic patterns of the serum and cerebrospinal fluid as well as their osmolality and water concentrations and 3) the distribution ratios of individual ions and osmolality between serum and cerebrospinal fluid. The acid-base balance of the blood is given in terms of its pH, whole blood buffer base concentration, its partial pressure of carbon dioxide and oxygen saturation (3, 4, 5, 6). The acid-base balance of cerebrospinal fluid will be given in terms of its pH, its bicarbonate concentration and partial pressure of carbon dioxide. The ionic patterns of both serum and cerebrospinal fluid are given in the form of ionograms and include the osmolality and water concentration of both. The ionic and osmolality distribution ratios are given in separate figures. Other pertinent data, not included in the foregoing, will be found in the discussion of the individual patient. This report is based on six patients, two with fracture of the skull, two with bullet-wounds of the head and one each with spontaneous intracerebral hematoma and pseudotumor cerebri.

METHODS AND MATERIALS

The mean values for the constituents of the blood, serum and spinal fluid used as controls were obtained on five male patients with a mean age of 45.2 years, range 39 to 61 years, who were undergoing myelograms to determine the presence of intraspinal pathology. Four of the five had completely negative myelograms, the other had evidence of a protruded intervertebral disc with a partial block to the passage of intrathecal

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PANTOPAQUE®. In all, the cerebrospinal fluid had a total protein concentration below 50 mgs. per cent, a negative globulin, a normal cell count and a normal gold curve and serology. The opening cerebrospinal fluid pressures were all below 150 mm. of water and the Queckenstedt maneuvers showed no evidence of an obstruction of the spinal pathway.

The spinal punctures were done in the lumbar region with the patient in the lateral position using an 18 gauge needle. Spinal fluid was withdrawn as near simultaneously as possible with blood sampling. Special attention was given to the maintenance of anaerobic conditions, temperature control, standardization of apparatus and rapidity of determination after sampling for the pH determination in both blood and cerebrospinal fluid (7, 8, 9). All pH determinations were made at 38°C.

Arterial blood and serum were used throughout. Sodium, potassium, calcium, magnesium, chloride, phosphorus, total carbon dioxide content of serum and cerebrospinal fluid, the hematocrit, oxygen saturation of the blood and total serum protein concentrations were determined by methods previously described (4, 5, 6). The pH of the blood and cerebrospinal fluid were determined as previously described (5) or by the Astrup technique (10). The protein of the cerebrospinal fluid was determined according to Merritt and Fremont-Smith (11). The hematocrit, blood total carbon dioxide content and pH were used to obtain the whole blood buffer base, partial pressure of carbon dioxide and the serum bicarbonate concentration using the nomogram of Singer and Hastings (3). The total carbon dioxide content and pH of the cerebrospinal fluid were used to calculate its bicarbonate concentration and partial pressure of carbon dioxide using the Henderson-Hasselbalch equation and pK' of 6.10 and α of 0.528 (12). Water concentrations were determined either by the procedure of Eisenman *et al.* (13) or Macdonald *et al.* (14). Osmolality of both fluids was determined by the Fiske osmometer standardized according to the recommendations of the manufacturer, bracketing between solutions of 100 and 400 mOsm./kg. water and applying the appropriate correction factors (15). Ionized serum magnesium and calcium concentrations were calculated from the nomograms of Willis and Sunderman (16) and of McLean and Hastings respectively (17). The inorganic phosphate partition was calculated by the Henderson-Hasselbalch equation using pK_2' of 6.80 and the observed pH (18).

For the calculation of the ionic distribution ratios the bicarbonate, chloride, and hydrogen ions (19) and the sodium and potassium of the serum have been considered freely diffusible and ultrafiltrable respectively (20). Inorganic phosphate of the serum is considered ultrafiltrable (21); however, studies on the passage of the two forms of serum inorganic phosphate through the red blood cell membrane indicate that the divalent ion is the form in which this ion migrates (22) and we have partitioned the inorganic phosphate into its two forms and used only the divalent ion to calculate the distribution ratio for inorganic phosphate. Serum bicarbonate has been considered freely diffusible (19); however a recent report indicates that bicarbonate passes from the blood to the spinal fluid slowly (23). We have calculated the distribution ratio of this ion on the basis that it is diffusible. The calculated ionized serum calcium and magnesium concentrations were used to calculate the distribution ratios of these ions (2). For calculation of the ionic distribution ratios, proper attention was given to concentrations expressed on the basis of water content, charge, valency and binding (2). The blood and serum pH have been considered equivalent.

The Acid-Base Balance of the Blood

As stated previously the acid-base balance pattern of the whole blood is given in terms of the blood pH, whole blood buffer base, $(B_{B^-})_b$, the

partial pressure of carbon dioxide, $P_{a_{CO_2}}$, and oxygen saturation, $S_{a_{O_2}}$. The basis for the description of the acid-base balance of the blood in these terms has been given (3, 4, 24, 25). In the clinical evaluation of the acid-base balance in patients with a variety of medical and surgical conditions we have found this formulation to be very helpful (4, 5, 6). These observations on each of the six patients and their mean values in our control group will be found in Table I. The values for some of the parameters in this control group differ from those previously reported from this laboratory as normal (4, 5). The mean whole blood buffer base of the control group is 48.2 mEq./L. as compared to the previously reported normal of 50.0 mEq./L.; similarly the mean partial pressure of carbon dioxide is 37 as compared to 40 mm. mercury, and the mean oxygen saturation is 92 as compared to 95 per cent. In the control group the mean blood pH, 7.42, is in agreement with our previously reported normal of 7.43 (4). The differences in partial pressure of carbon dioxide and oxygen saturation between this control group and our previously reported normal value may be due to the difference in ages of the subjects in these two groups. The previously reported normal values were obtained on a group of young normal males in their early twenties while the ages of this control group ranged from 35 to 61 years, mean 45.2 years. The partial pressure of carbon dioxide and oxygen saturation decrease with increasing age (26, 27). Observations in our laboratory indicate that the whole blood buffer base may decrease with increasing age (28). The range of the pH of the blood for the normal in our laboratory is 7.36 to 7.46 (5, 6). The concentration of the whole blood buffer base is used as a measure of the metabolic component concerned with the regulation of the acid-base balance of this fluid and the partial pressure of carbon dioxide as a measure of the respiratory component of these mechanisms.

Ionic Patterns, Osmolality and Water Concentrations of these Two Fluids

The ionic patterns of the serum and cerebrospinal fluids are given in the conventional form; however, concentrations are expressed in milliequivalents per kilogram of water of each fluid, Figures 1 through 6. Osmolality of each fluid is given in terms of milliosmoles per kilogram of the water content of that fluid, mOsm./kg. water, Figures 1 through 6.

Ionic and Osmolality Distribution Ratios

We have chosen to represent the relation of the individual ionic concentrations of serum and cerebrospinal fluid in terms of the Gibbs-Donnan equilibrium (2, 29). The hydrogen ion concentration of these two fluids was calculated from the determined pH which is the negative logarithm to the base ten of the hydrogen ion concentration. For uniformity we have expressed all these ratios in logarithmic form. A negative value for a

specific ratio signifies that the ratio was less than unity, and a positive value that it was greater than unity. The osmolality ratio was obtained by dividing the osmolal concentration of the cerebrospinal fluid by that in the serum. It will be noted that the range of the individual ionic distribution ratios is quite wide, Figures 7, 8, and 9. For this reason more significance is attached to a particular ratio which is outside this range than to one which is above or below its mean value (see discussion of ionic distribution ratios). Hereafter C.S.F. will be used to designate the cerebrospinal fluid.

Studies on Individual Patients: Clinical Status and Laboratory Observations

Patient V.P. was a 41 year old male who fell and sustained a fracture of the occipital bone. At operation on the day of injury the brain was severely contused and the intracranial pressure was increased. He remained in brainstem coma for over three years. At the time of the observations, 2 years and 3 months after the injury, he was in coma, receiving tube feedings and there were no complications. The acid-base balance patterns of the blood and C.S.F. revealed, as compared to the control, Table I and Figure 1: blood pH reduced, but within the normal range of 7.36 to 7.46, a slightly reduced whole blood buffer base, an increased partial

TABLE I

Acid-Base Balance of the Blood

Patient	Date	$(pH)_b$	$(B_B^+)_b$ mEq./L.	$P_{a_{CO_2}}$ mm.Hg	$S_{a_{O_2}}$ per cent	$(NPN)_b$ mg. per cent
Control		7.422	48.2	37	92	32
V.P.	3/18/59	7.372	47.7	44	89	37
A.D.	1/13/59	7.365	48.0	47	94	29
R.S.	1/19/59	7.455	50.0	40	90	32
	3/9/59	7.385	46.0	40	95	30
B.J.McL.	4/1/59	7.405	48.0	43	96	46
	4/7/59	7.380	48.0	46	95	64
	4/15/59	7.395	46.0	40	93	60
J.C.	2/24/59	7.350	44.0	42	94	35
	3/11/59	7.380	46.0	42	92	30
J.B.	6/12/58	7.375	47.0	41	93	34
	6/14/58	7.380	46.0	39	94	35
	6/18/58	7.350	46.0	46	90	40
	6/23/58	7.420	47.0	38	94	30

Values for the control group are the means. $(B_B^+)_b$ is the whole blood buffer base of arterial blood, $P_{a_{CO_2}}$ is the partial pressure of carbon dioxide of arterial blood, $S_{a_{O_2}}$ is the oxygen saturation of arterial blood. Subscripts b, s and CSF or f denote blood, serum and cerebrospinal fluid respectively.

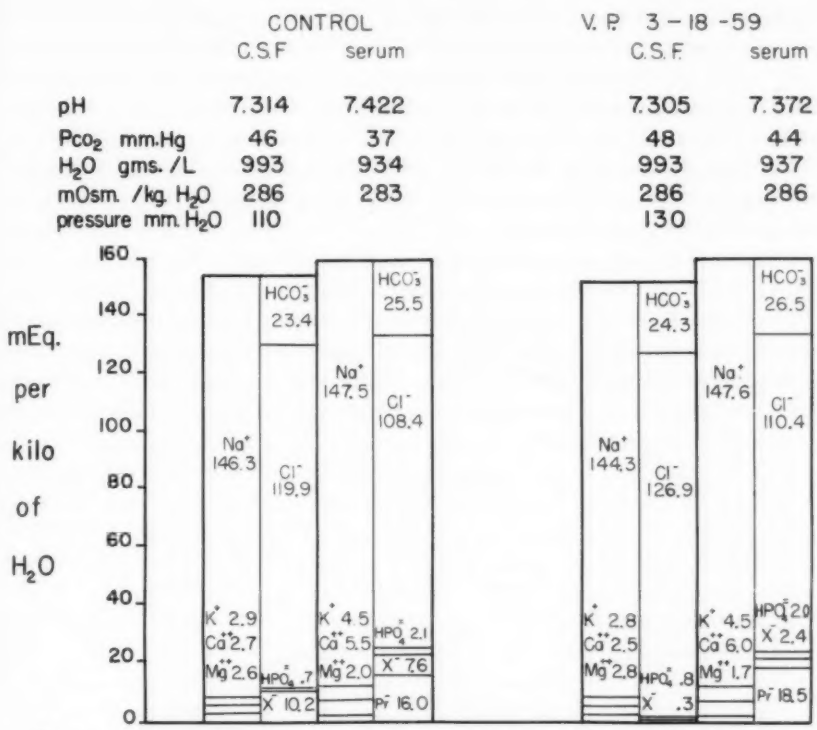

FIG. 1. A 41 year old male with a severe contused brain, injury sustained November 19, 1956, operation the same day. Studies performed more than two years later. C.S.F. denotes cerebrospinal fluid, P_{co_2} the partial pressure of carbon dioxide, Ca^{++}, Mg^{++} and $HPO_4^{=}$ are their total concentrations in this and Figure 2 through 6, mOsm. is the osmolality in milliosmoles/kg. of water, pressure is the opening pressure of the C.S.F.

pressure of carbon dioxide and decreased oxygen saturation, the pH of the C.S.F. was not reduced, its partial pressure of carbon dioxide and bicarbonate concentration were very slightly elevated. The ionic patterns of the two fluids, Figure 1, as compared to the control, revealed: a decrease in the undetermined anion concentrations of both and an increase of the chloride of the C.S.F. The osmolality of the serum was very slightly increased and its water concentration slightly elevated. The osmolality and water content of the C.S.F. were equal to their control values respectively. A comparison of the individual ion distribution ratios with their ranges in the control series, Figure 7, revealed: hydrogen and calcium were below, magnesium above and all the remainder were within their respective ranges. The osmolality distribution ratio was likewise

within its range for the control group. Thus the C.S.F. of this patient who had been in brainstem coma for over 2 years following a head injury revealed: an acid-base balance pattern similar to the control, its ionic pattern showed an increase in chloride, decrease in undetermined anion and very slight changes in cation concentrations, no changes in water concentration or osmolality and the ionic distribution ratios for hydrogen, calcium and magnesium ions were without their respective ranges for the control group.

Patient A.D., Figure 2 and Table I, was a 36 year old male who, two weeks prior to these studies, sustained a fracture of the left parietal region of the skull which extended into the middle fossa. Burrholes done shortly after admission disclosed an extensive subdural hygroma on the right and a contused brain on the left. He was confused, his dietary intake was good, and there was neither fever nor infection at the time of

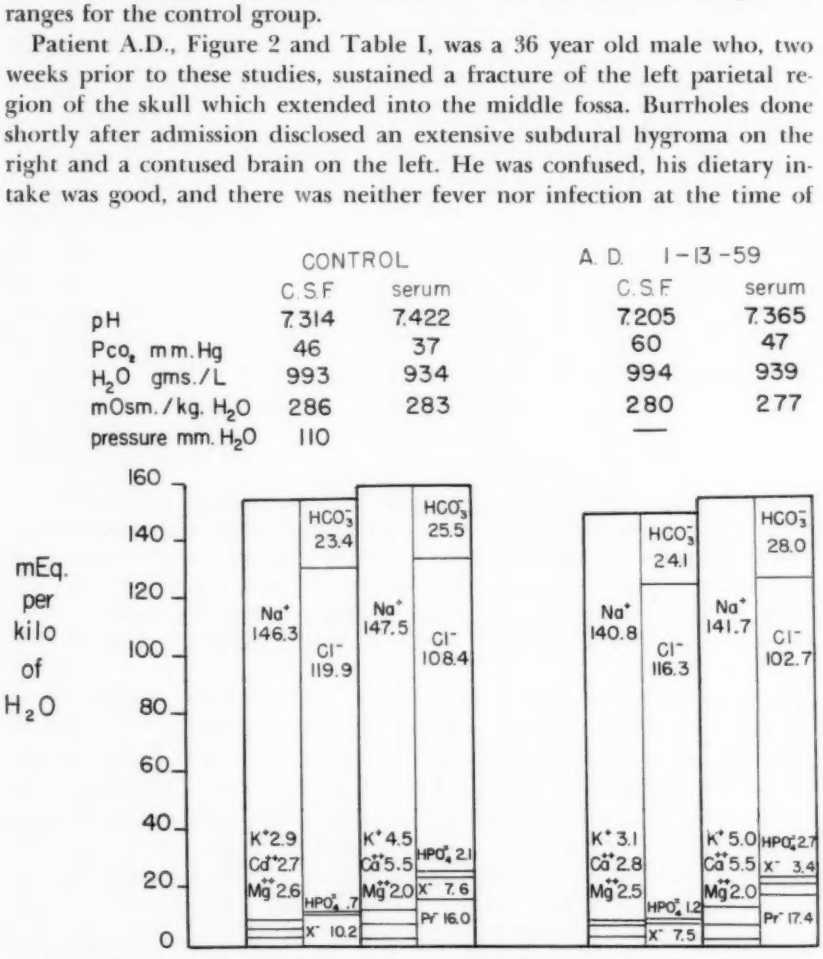

FIG. 2. A 36 year old male, fracture of left parietal region extending into the middle fossa, extensive subdural hygroma on right and contused brain on left, injury two weeks prior to these studies. For explanation of symbols see Figure 1.

the study. Parenteral fluids had not been administered during the preceding eleven days. He was discharged on his twenty-second postoperative day and one year later had completely recovered.

The acid-base balance of the blood as compared to the control group revealed, Table I: a reduced pH which, however, was within the normal range, a whole blood buffer base equal to the control, an increased partial pressure of carbon dioxide as well as oxygen saturation. A similar comparison of the acid-base balance of the C.S.F. revealed, Figure 2: a distinctly reduced pH, distinctly elevated partial pressure of carbon dioxide and a very slightly elevated bicarbonate concentration. Thus there was a tendency toward acidosis in the serum with an elevation of both of its partial pressure of carbon dioxide and bicarbonate concentration, the former more than the latter. Concomitantly there was a distinct acidosis of the C.S.F. with its bicarbonate concentration approximately equal to the control and a distinctly increased partial pressure of carbon dioxide.

The ionic patterns and osmolality of these two fluids, again as compared to the control, are depicted in Figure 2 and revealed: an increase in bicarbonate, potassium and water, a decrease in chloride, sodium, undetermined anion and osmolality concentrations of the serum; in the C.S.F. the chloride, undetermined anion, sodium and osmolality concentrations were below; the bicarbonate, inorganic phosphate and potassium were slightly above their control values, and its water concentration was one gram above the control. Thus the ionic patterns of both of these fluids differed from those in the control group and the decrease in the osmolality of the serum was accompanied by a distinct increase in water concentration of that fluid, but the decrease of osmolality of the C.S.F. was accompanied by a negligible increase in its water concentration.

On the basis of the range of the distribution ratios in the control series, Figure 7, the ratios of hydrogen, bicarbonate and calcium were above, those for inorganic phosphate and potassium were below, and those for chloride, sodium and magnesium ions and osmolality were within.

Patient R.S. was a 32 year old male who sustained a bullet wound of the right temporal region with the exit wound in the left frontal region; there was also a bullet wound of his left hand and severe frostbite of his ears and hands. He underwent primary debridement of his head and hand injuries. He was studied two weeks after the injury and again approximately two months later. The acid-base balance studies of the blood, Table I, revealed: an initially elevated blood pH which was within the normal range and subsequently decreased, the whole blood buffer base likewise decreased from its initial concentration, the partial pressure of carbon dioxide was 40 mm. of mercury at both samplings and there was an initially decreased oxygen saturation which subequently rose. The acid-base

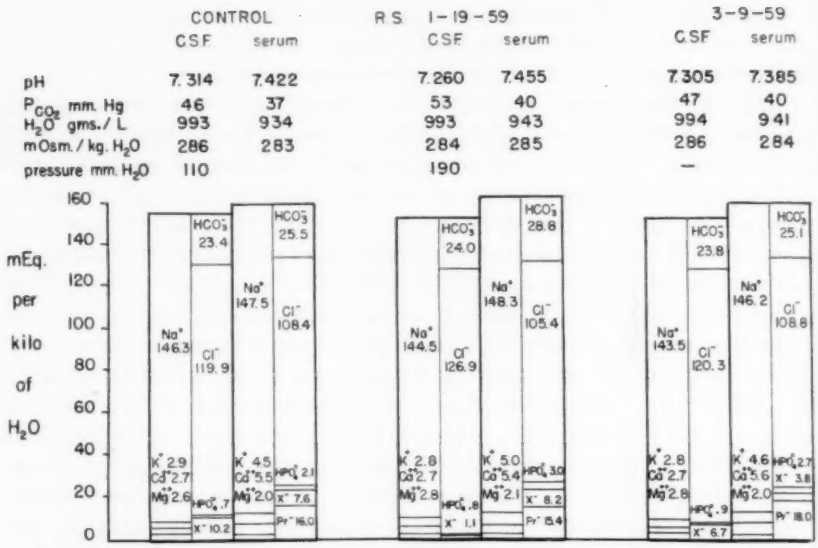

FIG. 3. A 32 year old male with gunshot wounds of the head, left hand and severe frostbite of ears and hands. Initial studies carried out two weeks following injury.

balance of the cerebrospinal fluid, Figure 3, revealed: an initial pH below the control which then rose to the control value, the partial pressure of carbon dioxide was initially above and then decreased to the control level and its bicarbonate concentration was consistently at the control level. Thus initially there was a tendency for alkalosis of the serum and a concomitant acidosis of the C.S.F., the latter due to an increase in the partial pressure of carbon dioxide without a decrease of its bicarbonate concentration. The initial acidosis of the C.S.F. was corrected on the second sampling by decreasing the partial pressure of carbon dioxide.

The ionic pattern of the serum, Figure 3, revealed initially, as compared to the control: an increase in bicarbonate, inorganic phosphate and potassium concentrations, a decrease in chloride concentration and the concentrations of sodium, calcium, magnesium and undetermined anion were approximately equal to the control. On the subsequent sampling only the inorganic phosphate concentration remained increased. The water of the serum was initially increased and then dropped but remained above its control level. The osmolality of the serum was approximately that of the control on both occasions. There was no increase in the water of the C.S.F. concomitantly with its increase in the serum. The osmolality of the C.S.F. was very near or at the level for the control throughout.

The ionic and osmolality distribution ratios, two weeks following injury, as compared to their respective ranges for the control series, Figure 7, revealed: the values for the hydrogen, bicarbonate and inorganic phosphate ions were above, those for chloride and potassium below, sodium, calcium and magnesium were within, and that for osmolality was below. Two months later calcium alone was above, potassium almost within and now hoydrogen, bicarbonate, chloride, inorganic phosphate, sodium, magnesium and osmolality were within their respective ranges. Thus initially five of the ionic ratios were above or below their respective ranges, and two months later all but one were within their respective ranges for the control series.

Patient B. J. McL. was a twenty-four year old male who sustained a gunshot wound over his left eye, Figure 4. He had a marked hemiparesis and aphasia, was incontinent of urine and feces and was receiving tube feedings. Debridement with extensive resection of necrotic brain and fractured bone over the frontal region and tracheotomy were accomplished. The studies were done, seven, thirteen and twenty-one days after the injury. The acid-base balance of the blood, Table I, revealed: pH between 7.38 and 7.41, whole blood buffer base of 46 to 48 milliequivalents per liter, partial pressure of carbon dioxide between 40 to 46 mm. of mercury, and an oxygen saturation ranging from 93 to 96 per cent. The nonprotein nitrogen of the blood was consistently elevated, 46 to 64 mg. per cent without evidence of acidosis. He was not on therapy with urea. The acid-base balance pattern of the C.S.F., Figure 4, revealed: an initial pH which was distinctly below the control and subsequently rose but remained consistently below the level for the control group, the partial pressure of carbon dioxide was initially increased and then decreased progressively but remained above the control, its initial bicarbonate concentration was below, then increased very slightly above, and finally dropped again below the control level. The C.S.F. revealed an initial acidosis which was accompanied by a decrease in its bicarbonate concentration and an increase in its partial pressure of carbon dioxide. This acidosis decreased thereafter and was associated with changes in both bicarbonate concentration and partial pressure of carbon dioxide.

The ionic pattern of the serum on the seventh day following injury revealed quantitative differences from the mean of the control group. In only one instance, potassium concentration, was this reflected in the C.S.F. as a change in concentration in the same direction, decrease, from the mean value of the controls. However this decrease of potassium concentration was not quantitatively equal in the two fluids, 0.1 milliequivalents per kilogram of water for serum and 0.6 milliequivalents per kilo-

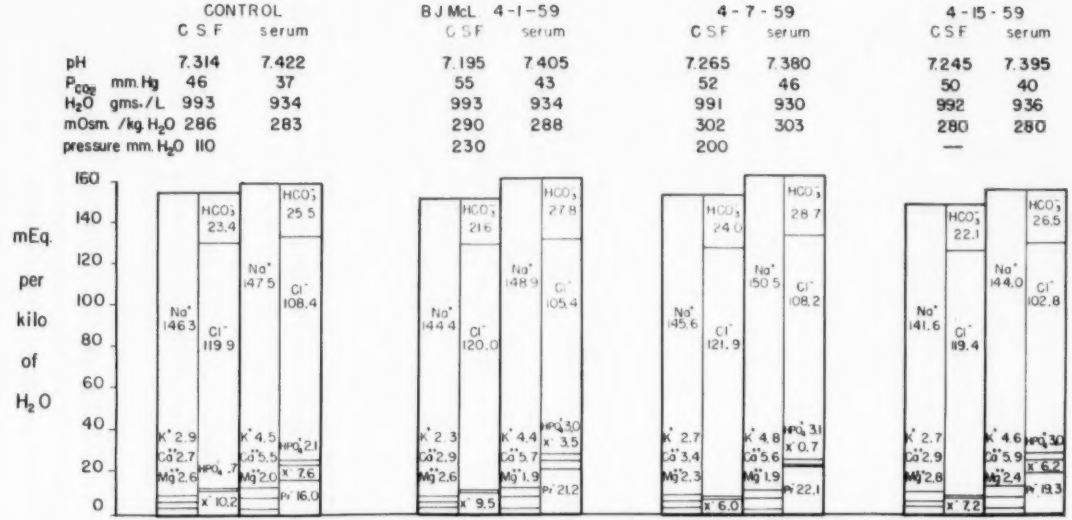

FIG. 4. Gunshot wound over left eye in a 24 year old male, marked hemiparesis and aphasia, studies done 7, 13 and 21 days following injury.

gram of water for C.S.F. On the next two observations the ionic concentrations were fluctuating and on both occasions these fluctuations were more frequently reflected in the C.S.F. as changes in concentration in the same direction, increases or decreases, in the two fluids. Again, only rarely were these reflections quantitatively equal.

The initial osmolality of both serum and C.S.F. was above the mean level of the controls. The initial water content of both fluids was that of the mean in the controls. The blood NPN was elevated, 46 mg. per cent. On the thirteenth day following injury the osmolality of both fluids increased, the water content of both decreased and the blood NPN had increased to 64 mg. per cent. By the twenty-first day following injury the osmolality of these fluids was below the initial as well as the control level and the water concentration of the serum was slightly elevated with a negligible increase in its concentration in the C.S.F., both as compared to their preceding levels.

The ionic and osmolality distribution ratios on the seventh, thirteenth and twenty-first days following trauma are given in Figure 8. As compared to the range of the individual ratios for the control series, hydrogen ion was initially above, then decreased to a value within and on the final sampling was slightly above it, the bicarbonate ratio was consistently above its range, chloride was within on the first two observations and below on the final observation, inorganic phosphate was initially above then dropped below and at the final observation was within. On the first two samplings the ratio for the sodium was below and that of the final sampling was within. The ratio for the potassium ion was consistently below, that for calcium consistently above and that for magnesium consistently within their respective control ranges. The osmolality ratio was consistently within its range for the control series.

Patient J.C. was a sixty-one year old male who sustained a spontaneous intracerebral hematoma of the right frontal lobe with profound coma and left hemiplegia, Figure 5. The intracerebral hematoma was removed and tracheotomy was performed. The first sampling was obtained exactly one month after the onset of the intracerebral hemorrhage at which time he was stuporous, on tube feedings and his temperature was normal. At the time of the second sampling he was speaking, some motion was present in his left leg and tube feedings were continuing. The initial studies of the acid-base balance of the blood, Table I, revealed a pH of 7.350 which is below our normal range and consequently he was considered to be in acidosis. The initial whole blood buffer base was reduced and the partial pressure of carbon dioxide was increased as compared to the control group. At a subsequent sampling, seventeen days later, the blood

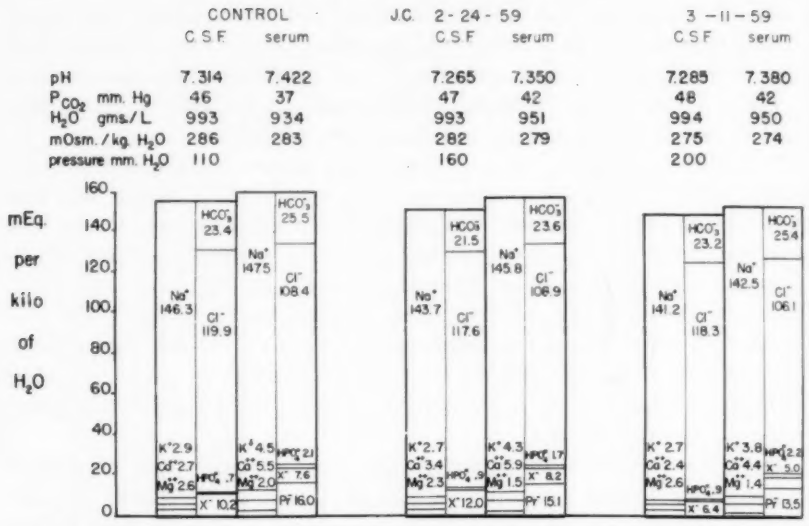

FIG. 5. A 61 year old male with a spontaneous intracerebral hematoma of the right frontal lobe, profound coma and left hemiplegia sustained one month prior to the initial observation.

pH and whole blood buffer base had increased while the partial pressure of carbon dioxide remained at its previous level and oxygen saturation had decreased slightly. The acid-base balance pattern of the C.S.F., Figure 5, at the first sampling with acidosis of the blood revealed: a pH below the control, a partial pressure of carbon dioxide approximately equal to the control and a diminished concentration of bicarbonate. Thus the acidosis of the C.S.F. was due to a diminished bicarbonate concentration and not due to an increase in partial pressure of carbon dioxide. At the second observation the pH of the serum and C.S.F. had both increased due to an increase in the bicarbonate concentration of each and without any change in the partial pressure of carbon dioxide of either as compared to the previous sampling. However, the pH of both of these fluids remained below their respective values in the controls. As compared to the control, the initial ionic pattern of the serum revealed: a decrease in bicarbonate, inorganic phosphate, sodium and magnesium, chloride and undetermined anion concentrations were approximately equal to and the calcium concentration was above. The initial pattern of the C.S.F. revealed a diminished bicarbonate, chloride and sodium, and an increase in calcium concentration as compared to the control series. Subsequently the bicarbonate concentration of both the serum and C.S.F. rose, the so-

dium concentration of both decreased, the inorganic phosphate of the serum increased without any change in its concentration in the C.S.F., the potassium of the serum decreased without any change in its level in the C.S.F., and the calcium concentration of both fluids decreased. The initial serum osmolality was decreased and its water concentration increased, the concomitant osmolality of the C.S.F. was reduced with a water content equal to the control. This pattern, a decrease in osmolality with an increase in water concentration of the serum and a concomitant decrease in osmolality without an increase in water concentration in the C.S.F., was again evident at the second sampling.

The distribution ratios compared to their respective ranges in the control group, Figure 7, revealed: hydrogen, bicarbonate, chloride, sodium ions and osmolality were consistently within their respective ranges, potassium was initially within and subsequently was noted to be above, that for inorganic phosphate was initially below and subsequently was within, and those for calcium and magnesium were consistently above their respective ranges.

Patient J.B., Figure 6, was a thirty-two year old male with pseudotumor cerebri who underwent arteriogram, pneumoencephalogram and right subtemporal decompression on June 12, 1958; the initial studies were carried out immediately prior to operation and further studies were done on the second, sixth and eleventh postoperative days. He had an episode of gout prior to operation and was treated with colchicine. There was a history of asthma, the anterior-posterior diameter of the chest was increased, the M.M.B.C. was slightly decreased and there was an obstructive element to his pulmonary gaseous exchange. He received 1000 ml. of 5 per cent dextrose in water and a similar volume of 5 per cent dextrose in normal saline during the first twenty-four hours following operation. There were no postoperative complications. The acid-base balance pattern of the blood, Table I, revealed: a blood pH which was within the normal range of 7.36 to 7.46 except on June 18, 1958 when it was 7.35 which is at the level of acidosis, the whole blood buffer base varied only 1 milliequivalent, 46 to 47 milliequivalents per liter of blood, the partial pressure of carbon dioxide varied from 38 to 46 mm. of mercury and oxygen saturation ranged from 90 to 94 per cent. The acidosis of the blood on June 18, six days following operation, was associated with an elevated partial pressure of carbon dioxide and a decrease in oxygen saturation, indicating a respiratory origin. The acid-base balance patterns of the C.S.F., Figure 6, revealed: a pH which was preoperatively approximately that of the control value and then decreased to reach its lowest value six days following operation, it then rose to reach the level of the

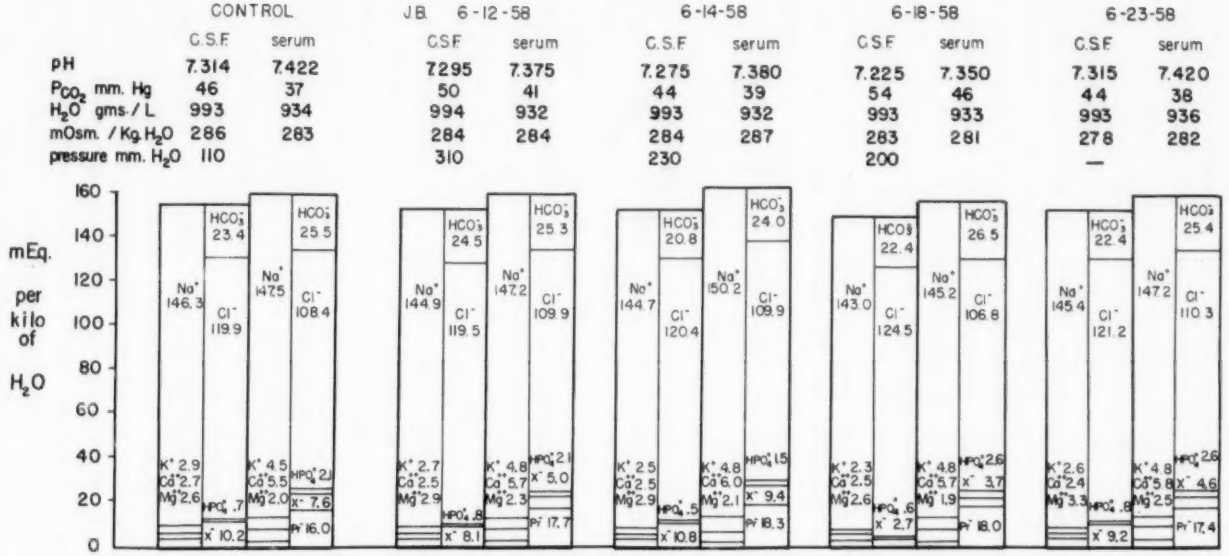

FIG. 6. A 32 year old male with pseudotumor cerebri who underwent arteriogram, pneumoencephalogram and right subtemporal decompression following the observations on June 12, 1958.

control by the eleventh postoperative day, the bicarbonate concentration dropped immediately postoperatively from an initial level which was slightly above the control and on the next two samplings it was slightly below its preoperative concentration, the partial pressure of carbon dioxide was initially slightly above the value for the control series, dropped immediately following operation only to rise on the sixth postoperative day to a level slightly above its initial value and on the last sampling it approached the control value. The lowest level of the pH of the C.S.F. occurred at a time when its partial pressure of carbon dioxide was at its highest, when its bicarbonate concentration had risen from its lowest value, and when the serum pH was at its lowest level. The initial serum electrolyte pattern was not that associated with a moderate or severe degree of pulmonary insufficiency, diminished pH, high bicarbonate and partial pressure of carbon dioxide and low chloride concentration (24) and this is in agreement with the mild degree of the changes in the pulmonary function studies in this patient with a history of asthma. The final acid-base balance pattern of the C.S.F., on the eleventh postoperative day, was essentially that of the control group.

The water concentration of the serum ranged from 932 to 936 gm. per liter, that of the C.S.F. varied only 1 gm., 994 to 993 gm. per liter, the osmolality of the serum ranged from 281 to 287 milliosmoles per kilogram of water, and that of the C.S.F. from 278 to 284 milliosmoles per kilogram of water.

The ionic and osmolality distribution rations are given in Figure 9 for each of the four samplings. The preoperative ratio for the hydrogen ion was within the range for the control series, then rose progressively for the first two samplings following operation, and at the final one was returning toward its preoperative value; however at no time did it exceed the range for the control group. The ratio for the bicarbonate ion followed a pattern similar to that for the hydrogen ion, however it did exceed the range for the control group on each of the postoperative samplings. The preoperative distribution ratio for the chloride ion remained within the range for the control group with the exception of the final observation which was below it. The inorganic phosphate ratio was preoperatively within this range, then rose progressively above it postoperatively for the two subsequent samplings, and finally decreased toward its preoperative level. The sodium distribution ratio was preoperatively within this range then decreased below it immediately postoperative, and on the last two samplings was within its control range. This ratio for the potassium ion was consistently below its control range, that for calcium fluctuated below and then within, and that for magnesium was consist-

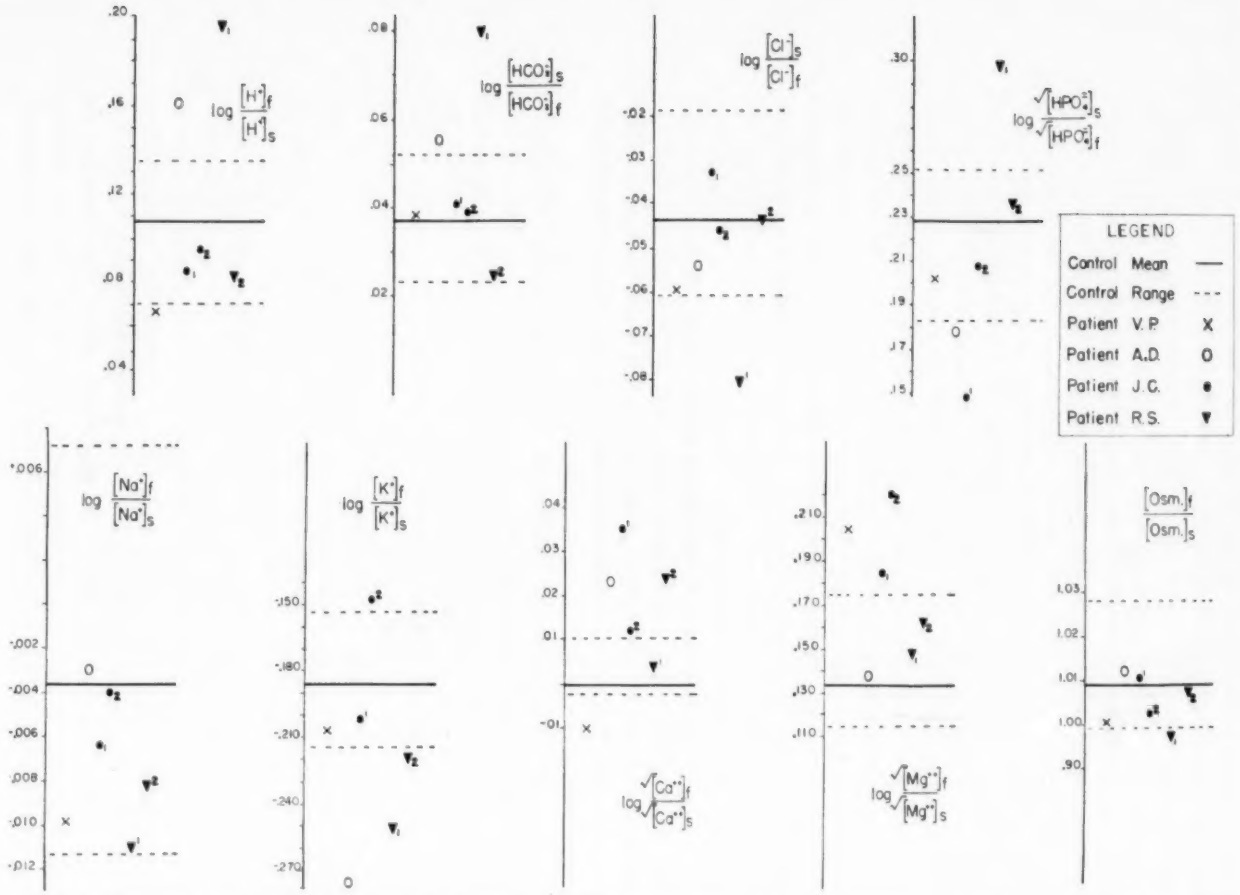

FIG. 7. The ionic and osmolality distribution ratios between serum and C.S.F. of patients V.P., A.D., J.C. and R.S., small numerals beside the symbols indicate the sequence of observations on J.C. and R.S., the method of calculating these ratios is given in the text.

FIG. 7. The ionic and osmolality distribution ratios between serum and C.S.F. of patients V.P., A.D., J.C. and R.S., small numerals beside the symbols indicate the sequence of observations on J.C. and R.S., the method of calculating these ratios is given in the text.

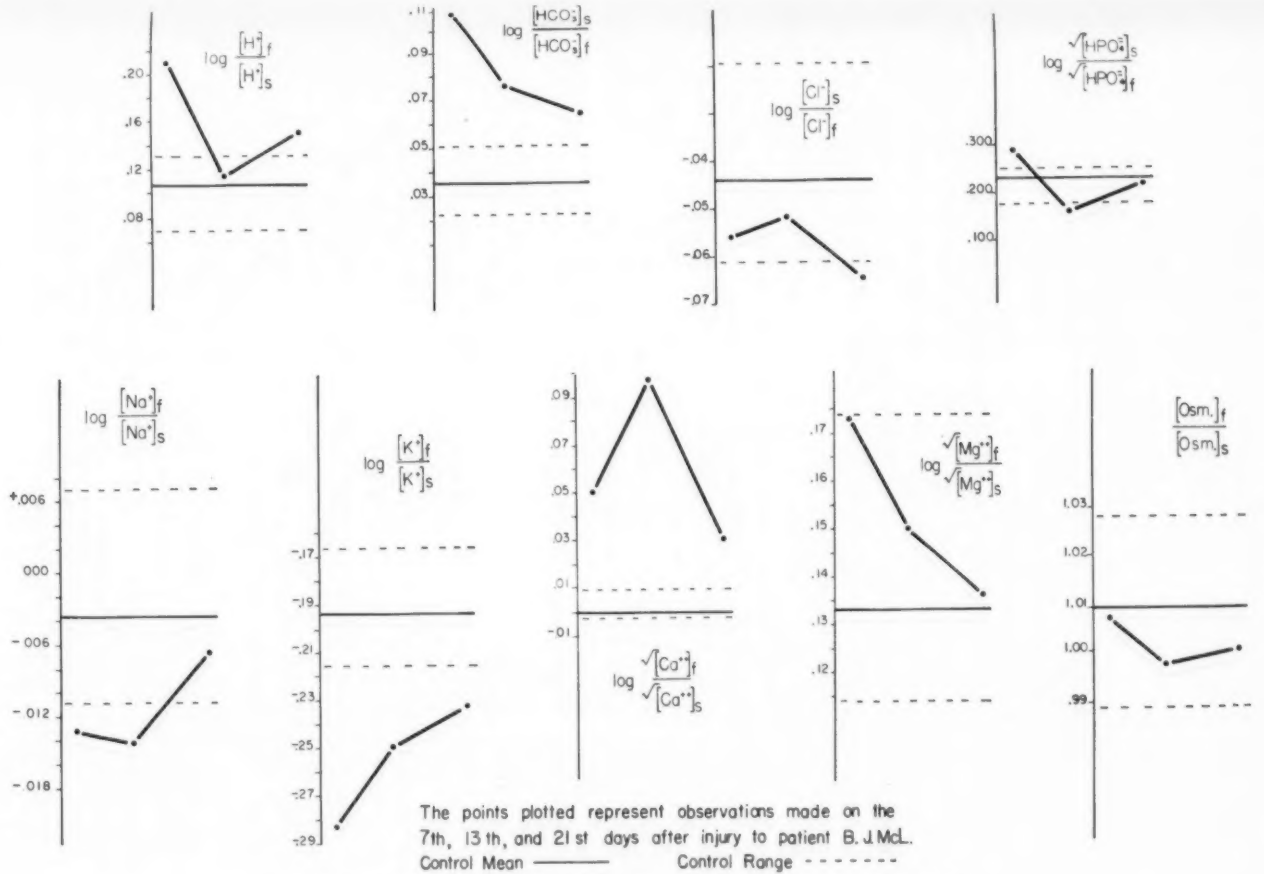

FIG. 8. The ionic and osmolality distribution ratios for patient B.J. McL.

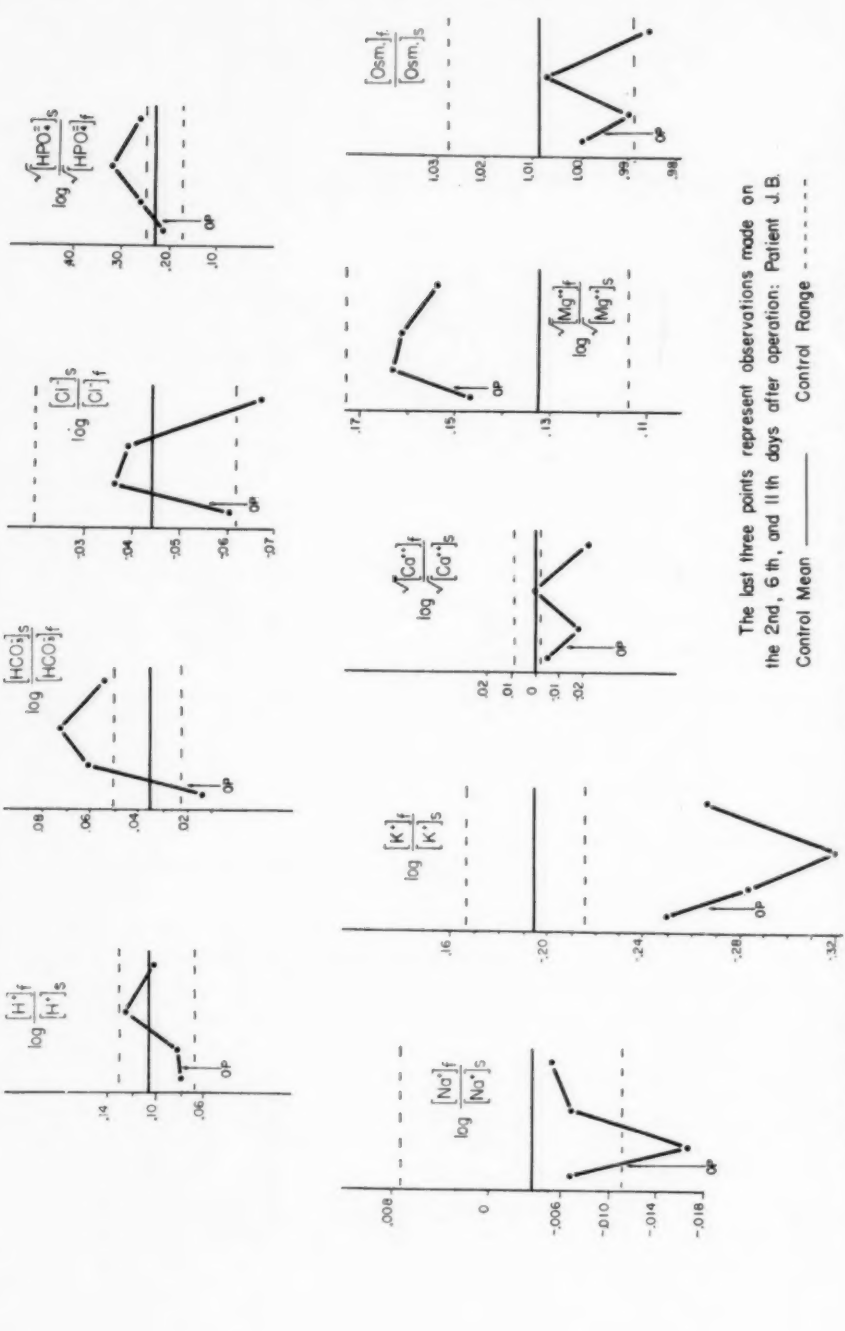
The last three points represent observations made on the 2nd, 6 th, and 11 th days after operation: Patient J.B.
Control Mean ——— Control Range - - - - - -
OP

FIG. 9. The ionic and osmolality distribution ratios between C.S.F. and serum of patient, J.B. OP indicates operation.

ently within. The osmolality distribution ratio was consistently within its control range with the exception of the final sampling which was below it.

FIG. 9. The ionic and osmolality distribution ratios between C.S.F. and serum of patient, J.B. OP indicates operation.

DISCUSSION

The ionic pattern of the C.S.F. in a normal adult man may be obtained from the literature (1, 2, 11, 30 to 39). Its acid-base balance pattern in terms of pH, bicarbonate concentration and partial pressure of carbon dioxide under similar conditions is not so readily available. Values for its pH from 7.13 to 7.95 have been reported (11, 30, 38). In our small control group this ranged from 7.285 to 7.335 with a mean of 7.314, and the simultaneously determined pH of the blood ranged from 7.419 to 7.440 with a mean of 7.422. The pH of the C.S.F. is below that of the blood. A recent report indicates this, mean serum pH 7.40 and mean pH of the C.S.F. 7.32 (30). In another report, using mentally retarded children as controls, the pH of the serum was 7.43 and that of the C.S.F. was 7.37 (40). Values for bicarbonate concentration of C.S.F. may be found which range from 21 to 28 milliequivalents per liter (2, 11, 36). The concentration of this ion in the C.S.F. of our control series ranged from 22.1 to 24.1 milliequivalents per liter with a mean of 23.2 milliequivalents per liter of C.S.F. The simultaneous concentration of bicarbonate in the serum ranged from 23.0 to 25.0 with a mean of 23.8 milliequivalents per liter of serum. The foregoing concentrations are per liter of each of these fluids and not as given in the figures, mEq./kg. of water. Bicarbonate contributes 68 per cent of the 34 milliequivalents per kilogram of water of the total buffer anion of the C.S.F. in contrast to 50 per cent of the 50 milliequivalents per kilogram of water of buffer anion of the serum. The bicarbonate-carbonic acid buffer system is the principal one in the C.S.F. The partial pressure of carbon dioxide of the C.S.F. has been reported as being 5 to 7 mm. of mercury higher than the serum (37). In our control series it ranged from 41 to 49 with a mean of 46 mm. of mercury. The simultaneous partial pressure of carbon dioxide of the serum ranged from 35 to 40 with a mean of 37 mm. of mercury. It has recently been pointed out that the acid-base relations of the C.S.F. differ from those of extracellular fluid generally (23, 41).

The pH of the C.S.F. in patient V.P., taken at more than two years following head injury, was equal to the mean of the control series. In the other patients with head injuries, the patient with spontaneous intracerebral hematoma and another with pseudotumor cerebri, following operation, the initial pH of the C.S.F. was below the mean value as well as the range of the control series, irrespective of whether the initial ob-

servation was made two days or one month following the insult. Furthermore, an initially decreased pH of this fluid increased with the elapsed time following the insult. The acute response to the types of trauma sustained by these patients is a lowering of the pH of C.S.F. which is normally below that of the serum. Subsequently this rises to its level in the control series. In no patient of this group was the acute response an increase in pH of the C.S.F. nor did the subsequent rise exceed the range of pH for the control series. This decrease in pH of the C.S.F. was due to a decrease in its bicarbonate concentration and/or an increase in its partial pressure of carbon dioxide. In J.B. on the second postoperative day the decreased pH was due to a decrease in bicarbonate concentration. In B.J. McL. on the thirteenth post-trauma day it was due to an increase in the partial pressure of carbon dioxide, and on the seventh and twenty-first post-trauma days both were present.

This relative acidosis of the C.S.F. was associated with the acidosis of the serum, pH below 7.36, in J.B. on the sixth postoperative day and in J.C. on the initial sampling. However the initial observation on R.S. revealed a pH of the C.S.F. which was below the mean of the control group and the simultaneous serum pH was at our upper limit for the normal, 7.46. The initially low pH of the C.S.F. of B.J. McL. was not accompanied by acidosis of the serum. Figure 10 depicts the relation of the pH of the C.S.F. to that of the serum for each observation. In this group of patients, there exists no single quantitative relation between the pH of these two fluids. This is in contrast to the relation of the osmolality of these fluids, to be considered subsequently. Acidosis has been described following head injury (42, 43) as well as alkalosis (44). Abnormalities of serum electrolyte concentrations have likewise been reported in such patients (42, 45 to 49).

To inquire into the control of pH in the C.S.F. of these patients we have selected three for a more detailed discussion. The acid-base balance of C.S.F., serum and blood is again described by the parameters used throughout this paper, and the whole blood buffer base and partial pressure of carbon dioxide of the blood are used as measures of the metabolic and respiratory components of the acid-base balance of this fluid respectively (3, 4, 24). The changes in the acid-base balance patterns of the C.S.F., with the simultaneous changes in the pH of the serum of these patients during the period of observation, will be compared to those in the same fluids of the experimental animal under similar conditions, metabolic or respiratory acidosis or alkalosis. The changes in the patterns in these patients are not as great as those encountered in the experimental animal. This quantitative difference need not prejudice our perspective.

Changes in the acid-base balance patterns of the C.S.F. which are similar or dissimilar to those of the experimental animal and produced by the same means are designated typical and atypical respectively.

In the experimental animal the production of acidosis or alkalosis of the serum by metabolic means produces changes in the pH of the C.S.F. which are in a direction opposite to those in the serum (23). This "paradoxical" shift is mediated by a change in the partial pressure of carbon dioxide of the C.S.F. without any change in its bicarbonate concentration (23). The experimental production of acidosis or alkalosis by respiratory means, administration of carbon dioxide-rich gas or hyperventilation, leads to parallel changes in the pH of serum and C.S.F. (23).

The changes in these patterns, during the period of observation, for R.S. and J.C. are given in Figure 11. In the former the changes of the pH of the serum and C.S.F. were reciprocal in contrast to those of the latter in whom the changes of pH were in the same direction in the two fluids. The whole blood buffer base of the former decreased, and that of the latter increased, and the levels of the partial pressure of carbon dioxide of both patients did not change with the changes in the pH of the blood, Table I. Therefore the changes in the pH of the serum in both were mediated by

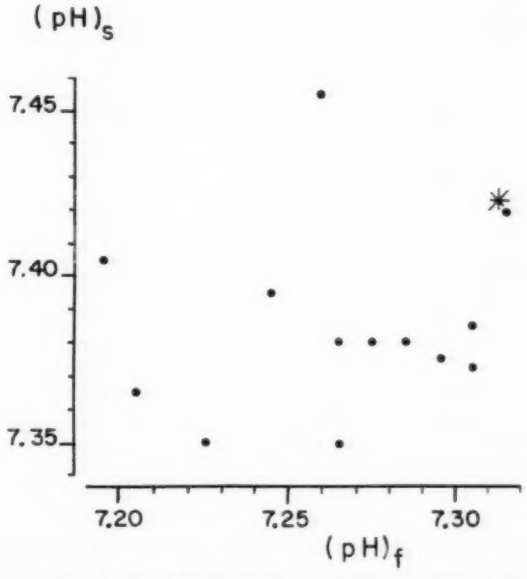

FIG 10. The relation between the pH of the serum and C.S.F. in the six patients, * denotes the mean value for the control group, subscripts s and f denote serum and C.S.F. respectively.

the metabolic component, pH changes of the serum produced by metabolic means. Consequently the expected change in pH of the C.S.F. and serum should be in the opposite direction in both patients. Such was the finding in R.S. but not in J.C. In the former the increase in the pH of the C.S.F. with a simultaneous decrease in pH of the serum and the decrease in partial pressure of carbon dioxide of the C.S.F. without any change in its bicarbonate concentration are typical for experimental metabolic acidosis. In the latter patient the increase in both the pH of the serum and the C.S.F., and the increase of bicarbonate concentration without change of

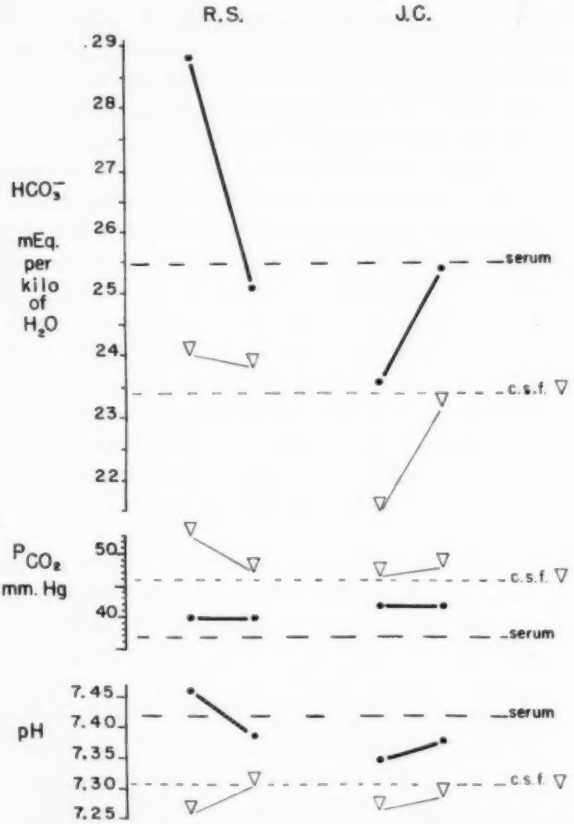

FIG. 11. The acid-base balance patterns of the serum and C.S.F. of patients R.S. and J.C. during the period of observation as given in the text. The solid circles are serum values, the open inverted triangles are those for the C.S.F.; the dark horizontal interrupted lines are the mean values of a constituent for the control group; the light horizontal interrupted lines are those for the C.S.F.

the partial pressure of the C.S.F., are atypical of experimental metabolic alkalosis.

The acid-base balance patterns of the serum and C.S.F. of J.B. who underwent operation for pseudotumor cerebri are depicted in Figure 12, prior to and on the second, sixth and eleventh days following operation. This patient's pattern in the C.S.F. is more complex than that of the two previous patients. In R.S. the bicarbonate concentration of the C.S.F. did not vary with the decrease in its partial pressure of carbon dioxide. In J.C. the partial pressure of carbon dioxide of the C.S.F. remained fixed and its bicarbonate concentration increased. In this third patient the partial pressure of carbon dioxide of the C.S.F. varied con-

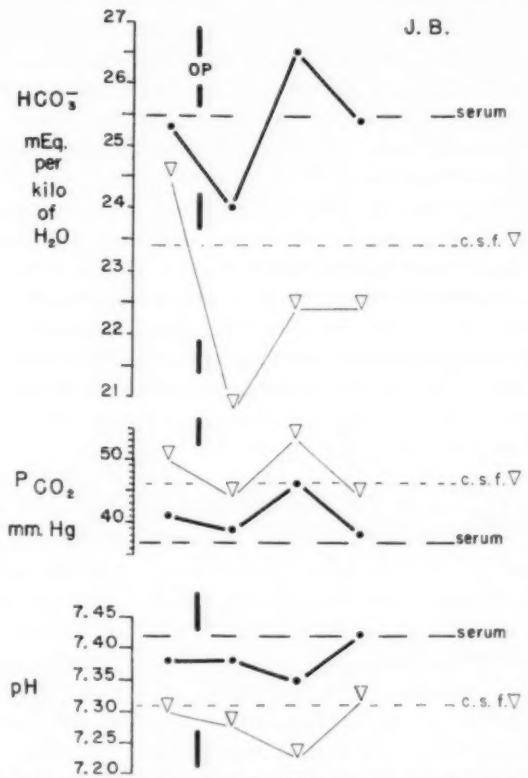

FIG. 12. The acid-base balance patterns of the serum and C.S.F. in J.B. prior to and following operation. The dark, vertical, interrupted line, marked OP, denotes the day of operation, the initial observations were taken immediately prior to operation, those subsequently at the second, sixth and eleventh day following operation. For further explanation see legend of Figure 11.

tinuously and its bicarbonate concentration decreased, increased, or did not change. Furthermore the rôle of this patient's impaired pulmonary function, see case presentation, in the maintenance of the acid-base balance of the blood becomes evident in the changing partial pressure of carbon dioxide and oxygen saturation of the blood on the third and fourth observations, Table I. The decrease of the pH of the blood between the second and sixth postoperative days, and its increase between the sixth and eleventh postoperative days, were due to an increase or decrease of the partial pressure of carbon dioxide without any change in the whole blood buffer base concentration, Table I. Consequently these pH changes of the serum were mediated by respiratory and not metabolic means. Therefore the pH changes of the C.S.F. and serum were in the same direction on these two samplings, Figure 12. These observations on J.B., as well as those on R.S., demonstrate that the pH of the C.S.F. in this group of patients can be affected by both the metabolic and respiratory mechanisms which regulate the acid-base balance of the blood, and that the resulting patterns of the C.S.F. are qualitatively similar to those of experimental metabolic and respiratory acidosis or alkalosis.

The pH of the serum of J.B. on the second postoperative day did not change from its preoperative level; however, there was a decrease in pH of the C.S.F. between these samplings, Figure 12, due to a diminution of 3.7 milliequivalents per kilogram of water of bicarbonate in the C.S.F. This pattern of pH in the two fluids has been classified as atypical. In the immediate postoperative period the bicarbonate concentration of the C.S.F. determined its pH and subsequently on two occasions its partial pressure of carbon dioxide was the controlling factor, decreasing it on one occasion and increasing it on another.

The atypical pattern of the acid-base balance of the C.S.F. in J.B. on the second postoperative day and that in J.C., Figure 11, indicate that the pH of the C.S.F. can be affected by factor(s) in addition to acidosis or alkalosis in the blood. The rôle of the distribution ratio of bicarbonate will be considered following the discussion of the ionic distribution ratios.

The mean values of the ionic distribution ratios, in logarithmic form, for the control series were: hydrogen 0.108, bicarbonate 0.037, chloride −0.044, divalent inorganic phosphate 0.023, sodium −0.004, potassium −0.194, calcium 0.000 and magnesium 0.133. For the same group the osmolality distribution ratio was 1.011. Average ionic distribution ratios across collodion or capillary membranes reported in the literature and expressed as logarithms are: bicarbonate −0.002, chloride −0.007, sodium −0.040, potassium −0.119, calcium −0.102, and magnesium −0.061 (2). Our ratios and those reported are not in agreement and we attribute this difference to the conditions under which the two sets were determined.

In Figures 7 through 9 it will be noted that the range of the ratio for a specific ion in the control series may be wide, e.g. sodium, and that for another may be narrow, e.g. calcium. This finding is analogous to the marked variation in the sodium exchange time in the C.S.F. of dogs (50) and is probably due to many independently operating factors which affect permeability (50). The barrier separating serum and C.S.F. is composed of a number of structures, the capillary endothelium, ependyma, pia-glial membranes, choroid plexus and arachnoid membrane, each of which probably has different permeability characteristics (50).

The distribution ratios of the individual ions may or may not vary in a similar manner in a single patient. The changes in the bicarbonate and chloride ratios follow a similar pattern and those for potassium are distinctly dissimilar in J.B., Figure 9. Likewise the changes of the distribution ratio for the same ion may or may not be similar from patient to patient. The change in the ratio for sodium in J.B., and B.J. McL. are similar, and those for potassium are dissimilar in the two patients, Figures 8 and 9. The types of trauma sustained by these patients do not affect all the ionic distribution ratios in a similar manner. This is not surprising when the nature of the barriers separating serum and C.S.F. are considered (50). A particular ratio may be outside the range of the control group initially following trauma and with the lapse of time, possibly associated with recovery, it will be found to be within this range, observations on R.S., Figure 7, and J.B., Figure 9.

The rôle of the ionic distribution ratio of bicarbonate in the acid-base balance of the C.S.F. is derived as follows:

1. $$pH_f = 6.10 + \log \frac{[HCO_3^-]_f}{0.0312\,P}$$

The Henderson-Hasselbalch equation for the bicarbonate-carbonic acid buffer system of the C.S.F. where P is the partial pressure of carbon dioxide.

2. $$r = \frac{[HCO_3^-]_s}{[HCO_3^-]_f}$$

the Gibbs-Donnan equilibrium for the bicarbonate of the serum and C.S.F. where r is the distribution ratio or

$$[HCO_3^-]_f = \frac{[HCO_3^-]_s}{r}$$

by substitution,

3. $$pH_f = 6.10 + \log \frac{\frac{[HCO_3^-]_s}{r}}{0.0312\,P}$$

TABLE II

Acid-Base Balance of the C.S.F. of Three Patients

Patient & Observation	$[HCO_3]_f$ mEq./kg.H_2O	$[HCO_3]_s$ mEq./kg.H_2O	r	PCO_{2f} mm.Hg	pH_f		Type of Change in pH of C.S.F.
					obs.	calc.	
Control, mean	23.4	25.5	1.089	46	7.314	7.315	
R.S.							
1	24.0	28.8	1.200	53	7.260	7.261	Typical experimental metabolic acidosis
2	23.8	25.1	1.050	47	7.305	7.312	
J.C.							
1	21.5	23.6	1.097	47	7.265	7.266	Atypical experimental metabolic alkalosis
2	23.2	25.4	1.095	48	7.285	7.289	
J.B.							
1	24.5	25.3	1.033	50	7.295	7.296	Atypical, no change in pH of serum
2	20.8	24.0	1.154	44	7.275	7.279	
3	22.4	26.5	1.183	54	7.225	7.223	Typical experimental respiratory acidosis
4	22.4	25.4	1.134	44	7.315	7.313	Typical experimental respiratory alkalosis

r is the ionic distribution ratio of bicarbonate; ob. is observed; calc. is calculated; typical and atypical are defined in the text; subscripts f and s denote C.S.F. and serum respectively.

From the third equation it is apparent that the pH of the C.S.F. can be influenced by the bicarbonate concentration of the serum, the ionic distribution ratio for bicarbonate and the partial pressure of carbon dioxide of the C.S.F. in addition to its bicarbonate concentration and the partial pressure of carbon dioxide as set forth in the first equation. In Table II will be found the data necessary to use the third equation as well as the observed and calculated pH of the C.S.F., using the equation, and the types of change in the pH of the C.S.F. of the three patients. The validity of this equation is found in the excellent agreement of the observed and calculated values for the pH of the C.S.F. given in this table.

The typical pattern of the acid-base balance of the C.S.F. in metabolic acidosis or alkalosis, pH shift opposite in direction to that in the serum and a change in the partial pressure of carbon dioxide without any change in bicarbonate concentration, requires the ionic distribution of bicarbonate to change. Such were the findings in R.S., this distribution ratio decreased from 1.200 to 1.050, Table II. When this ratio remains constant under these conditions the acid-base pattern of the C.S.F. will not conform to the experimental findings. In J.C. the pH changes in C.S.F. were atypical of metabolic alkalosis and the ratio remained constant, 1.097 and 1.095. In the first two observations on J.B., the bicarbonate concentra-

tion and partial pressure of carbon dioxide of the C.S.F. as well as the distribution ratio of bicarbonate were all changing and the resulting change in the pH of the C.S.F. was atypical, no change in the pH of the serum and a decrease in that of C.S.F. The typical changes of respiratory alkalosis in the C.S.F. of J.B., fourth observation Table II, are associated with a distribution ratio for bicarbonate which decreased from 1.183 to 1.134. Furthermore, the third equation permits the calculation of the pH of the C.S.F. when the bicarbonate concentration of the serum, the partial pressure of carbon dioxide of the C.S.F. and the distribution ratio for bicarbonate are all varying as they do in J.B. The findings in the foregoing three patients demonstrate the rôle of the distribution ratio for bicarbonate in the acid-base balance of the C.S.F.

The osmolality of the C.S.F. varied from 275 to 302 milliosmoles per kilogram of water and that of the serum from 274 to 303 milliosmoles per kilogram of water. The range of variation was the same in the two fluids. The variation of osmolality of the C.S.F. with that of the serum for each observation is given in Figure 13. This data can be fit into a one to one relation, $(Osm)_f = (Osm)_s$. This relation is also evident from the small variation of the distribution ratios for osmolality from unity, Figures 7, 8, and 9. An increase or decrease of the osmolality of the serum from the

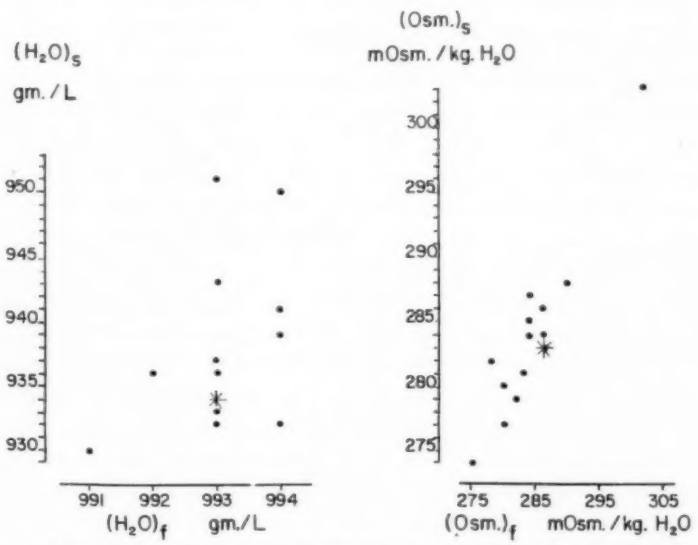

FIG. 13. The relation of the water of the serum and C.S.F. (left) and that of the osmolality of these fluids (right) for all the observations on the six patients,* represents the mean values for the control group.

mean control value is accompanied by a change in the osmolality of the C.S.F. in the same direction and of equal magnitude. As measured by the depression of the freezing point, the osmolalities of the C.S.F. and the serum in the normal have been considered equal (2, 11). In this group of patients the osmotic equality of these two fluids have been maintained over a range of 274 to 303 milliosmoles per kilogram of water. This relation is maintained during changes of osmolality effected by changes in ionic concentrations as well as an increase in urea concentration as measured by the NPN of the blood, observations on B.J. McL., Table I and Figure 4.

The water concentration of the C.S.F. varied from 991 to 994 gm. per liter and that of the serum from 930 to 950 gm. per liter. These simultaneous observations are depicted in Figure 13. The water concentration of the C.S.F. varied 3 gm. per liter while that of the serum varied 21 gm. per liter, a seven-flold variation in serum as compared to C.S.F. Furthermore all but two of the observations on the C.S.F. were within the range of 993 to 994 gm. per liter. The water content of the C.S.F. is held within relativley narrow limits with changes in the concentration of water in the serum. Four observations in this figure appear to have a straight line relation; however, this is due to the scale-size used and no single equation will fit all the data.

SUMMARY

The acute response in the acid-base balance of the C.S.F. to the types of trauma sustained by the patients in this report was a lowering of its pH which is normally below that of the blood. Subsequently this rose to the normal level. This decrease in pH of the C.S.F. was due to a decrease of its bicarbonate concentration and/or an increase in its partial pressure of carbon dioxide. The acid-base balance patterns of the C.S.F. of these patients were affected by both the metabolic and respiratory components of the acid-base balance of the blood. However, these were not the sole determinants. The ionic distributions ratio of bicarbonate between serum and C.S.F. was a modifying factor. C.S.F. and serum remained in osmotic equality, as measured by osmolality, over a range of 274 to 303 milliosmoles per kilogram of water in these patients. In this group the water concentration of the C.S.F. was relatively stable during variation of its concentration in the serum from 930 to 951 gm. per liter. All the individual ionic distribution ratios between C.S.F. and serum were not affected in the same manner nor degree by the types of trauma sustained by these patients.

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AN UNUSUAL CASE OF PARADOXIC EMBOLISM AFTER CARDIAC SURGERY

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Paradoxic embolism is the transport of a thrombus or other embolic material through an abnormal communication from the venous to the arterial circulation. Since the embolus can rarely be seen passing through the defect, such a diagnosis must generally remain presumptive. Cohnheim in 1877 (1) was the first to report an autopsy on a patient who probably had this type of embolism. In 1881 Zahn (2) demonstrated an embolus lodged in a patent foramen ovale and thus recorded the first case in which the diagnosis cannot be doubted. Since then 51 further such indisputable instances of paradoxic embolism have been reported (3 to 8). In almost all of these the embolus lay *in situ* in the foramen ovale.

Embolic phenomena are a well recognized complication of cardiac surgery (9). This report describes a patient who developed a most unusual type of paradoxic embolism after a cardiac operation.

CASE REPORT

This baby girl was born after a normal pregnancy and appeared well immediately after birth. Cyanosis, however, developed gradually during the second week of life. Otherwise, her early mental and physical development were normal. When she started walking, exertional dyspnea and tachycardia were noted by her parents. Squatting was not observed at any time.

Examination at the age of three years showed mild tachypnea without dyspnea, moderate cyanosis of the lips and nail beds, and some clubbing of fingers and toes. She was below normal weight for her age but of average height. Her pulse rate was 140 per minute, blood pressure 98/59 mm. of mercury and respirations 30 per minute.

There was mild precordial prominence and cardiac enlargement. No thrill could be felt. A grade two systolic murmur was heard maximally in the third left intercostal space. It was transmitted to the right of the sternum, to the arteries in the neck, to the left axilla, and to the left interscapular area posteriorly. No diastolic murmur was heard. All arterial pulsations were present and normal. There was no venous engorgement, edema, or hepatomegaly; the remainder of her physical examination showed no abnormalities.

The hemoglobin was 15.6 Gm. per cent. The electrocardiogram showed right axis deviation and a right ventricular strain pattern. On radiography there was right ventricular enlargement, a normal pulmonary conus, and diminished pulmonary vascularity.

A diagnosis of pulmonic stenosis with a right to left shunt was made. Cardiac catheterization confirmed this diagnosis and localized the shunt to the atrial level.

It was decided to perform surgical correction by open cardiotomy, the circulation

being maintained with a cardiopulmonary by-pass. The pulmonic stenosis was found to be due to valvular stenosis and diffuse muscular hypertrophy of the right ventricle. The valvular stenosis was corrected by incision of the three fused commissures, and the out-flow tract was enlarged by resection of a small amount of cardiac muscle. Subsequently the atrial defect consisting of a patent foramen ovale was sutured. Until this opening was sutured a catheter previously placed in the left atrium intermittently aspirated air.

The immediate postoperative course was satisfactory except for slight lethargy. The patient could answer questions and move all of her extremities. On the morning of the second postoperative day, however, she was unable to support herself when sitting up and could not move her left arm and leg. There was an extensor plantar response on the left side. Ophthalmodynamometry showed the retinal arterial pressures to be approximately equal in both eyes, suggesting absence of obstruction proximal to the origin of the ophthalmic artery. Nevertheless, because of the onset of hemiplegia after a lag period, a right-sided cerebral embolus was diagnosed. Her lethargy increased and she developed

FIG. 1. The embolus, composed of ventricular myocardium lodged in the internal carotid artery. (Hematoxylin and eosin ×30).

bradycardia and rigidity of her lower limbs. Dilatation of the pupils developed, particularly the right one, which did not react to light. Later she became deeply comatose. In spite of tracheostomy, hypothermia, bitemporal decompression and intravenous urea she died on the sixth postoperative day.

At autopsy the heart weighed 150 Gm. There was striking hypertrophy of the right ventricle and of both atria. The atrial septal defect in the position of the foramen ovale was closed by a suture line 2 cm. in length. The right atrial surface of this closed defect was roughened by some adherent tan thrombus material. The corresponding left atrial surface was smooth. The myocardium of the anterior wall of the infundibulum was reddish yellow, roughened and thinned where ventricular muscle had been resected to correct the infundibular stenosis. The pulmonic valve leaflets were thickened and showed only slight residual fusion at their commissures.

There was generalized cerebral edema with swollen flat gyri and tonsillar and uncal herniations. The edema was particularly marked on the right side where almost the entire cortex was softened and hemorrhagic. At the emergence of the right internal carotid artery from the dura it was filled by a cylinder of firm brown material measuring $0.7 \times 0.4 \times 0.3$ cm. This extended to the bifurcation of the artery into anterior and middle cerebral arteries. Histologically this brown material consisted of partly necrotic myocardium (Fig. 1). The size of the individual fibers, the arrangement of the muscle bundles and the presence of several small arteries all indicated that the embolic material was ventricular muscle.

DISCUSSION

The child's death was undoubtedly due to postoperative embolism of the right internal carotid artery. Grossly the embolus was thought to consist of thrombotic material. To our surprise histologic examination showed it to be ventricular muscle. Since the left ventricle was completely unscathed the only possible source of this embolus was the infundibulum where the right ventricular outflow tract was widened at operation. As there was no other communication between the pulmonary and systemic circulations, the embolus must have entered the left side of the heart through the foramen ovale before this opening was closed surgically. Paradoxic embolism, therefore, took place during the operation. It is difficult to explain why no symptoms were observed until 24 hours after operation. The obstruction of the internal carotid artery may have been only partial at first. Alternatively one might speculate that the collateral circulation in the circle of Willis delayed infarction while this cylinder of myocardial tissue was arrested in the carotid syphon. A subsequent change in the position of the embolus to the bifurcation of the internal carotid artery would soon be followed by hemiplegia and cerebral edema.

No previous record of this extraordinary type of paradoxic embolism has been found. Closure of the septal defect before widening the right ventricular outflow tract might have prevented this strange sequence of events.

SUMMARY

Paradoxic embolism after surgical correction of an atrial septal defect with pulmonic stenosis is reported. The embolus consisted of partly necrotic ventricular myocardium.

ACKNOWLEDGMENT

We wish to thank Dr. D. B. Clark for his helpful comments on the clinical course.

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ABSTRACTS OF PAPERS PRESENTED ON STUDENT RESEARCH DAY, MARCH 15, 1961

EXPERIMENTAL STUDIES WITH THE BUTANOLAMIDE AND PROPANOLAMIDE OF 1-METHYL-LYSERGIC ACID

MARTIN GELFAND

Two derivatives of 1-methyl-lysergic acid, the butanolamide (UML) and propanolamide (PML), were confirmed as being antagonistic to serotonin (5-HT). This was demonstrated by: 1) inhibition of the edema in a rat's paw, normally provoked by the local administration of a small dose of 5-HT or compound 48/80, a releaser of both 5-HT and histamine, but not that caused by a large dose of histamine alone; 2) decrease in the acute toxicity of 5-HT administered IP to mice but no effect on the mortality due to histamine; 3) diminution of the anaphylactoid reaction, a natural hypersensitivity in the rat mediated by the release of 5-HT.

Both anti-serotonins significantly diminished the toxicity due to anaphylaxis in mice. This is important evidence for the role of 5-HT in mediating anaphylaxis in this species.

As yet, it is unproven that serotonin has any normal function in the human body. Perhaps, in the evolution of man, serotonin has become a biochemical "appendix", and it may be no mere coincidence that this vestigial organ contains a large number of enterochromaffin cells that produce this substance.

NATIVE STATE OF THE OXYTOCIC HORMONE IN THE POSTERIOR PITUITARY GLAND

RICHARD E. SMITH[1]

Earlier studies on sedimentation in the ultracentrifuge indicated that the oxytocic hormone in its native state resides in the gland as a large molecular species. The present study, utilizing chromatographic analysis, corroborates the large molecular state of the natural hormone and further, reveals a new oxytocic unit of a molecular size intermediate between the octapeptide and the van Dyke protein. Application of dialyzed press-juice to a CM-cellulose column preceded gradient elution, with pH increasing from 6.0 to 7.5 and NaCl increasing from 0 to 0.5 M. Three discrete zones of oxytocic activity appeared in the chromatogram. Measurements on the rate of transfer of activity across a cellophane membrane under pressure filtration provided an estimate of molecular size. Peak 1 material, 80 per cent to 90 per cent of total activity, filtered at the slow rate of a protein; peak 2 activity, 10 per cent of total, traversed the membrane at the same rapid rate as synthetic octapeptide; peak 3 activity exhibited an intermediate filtration rate. Peak 2 octapeptide may be generated in the column by dissociation of peak 1 protein. The peak 3 material seems to be a natural constituent of the gland.

HYALURONIDASE ACTIVITY OF THE URINE FOLLOWING PITRESSIN INJECTION

R. J. CORRY AND R. E. COOKE

Since Ginetzinsky's discovery of hyaluronidase activity in concentrated urine of high osmolality considerable interest has been shown in regard to the reproducibility

[1] Denison Scholar for 1960–61.

of his results. The interpretation of his findings is that antidiuretic hormone (ADH) acts on the distal and collecting renal tubular cells to effect a release of hyaluronidase which in turn depolymerizes the intercellular hyaluronic acid matrix. This depolymerization, he states, increases the permeability of the intercellular material to water which moves from the tubular lumen into the hypertonic interstitial region thereby concentrating the tubular fluid.

The experiments to be reported were designed in an effort to reproduce Ginetzinsky's results and to examine the temporal relationship of pitressin administration to urinary hyaluronidase.

Hyaluronidase activity in the urine was determined by Ginetzinsky's viscosity reduction method. Two 90 second viscosimeters were placed in a constant temperature water bath at 37°C. The composition of the fluid to be investigated consisted of 2.67 ml. of a 200 mg. per cent solution of hyaluronic acid substrate, 0.67 ml. of citrate buffer, and 0.67 ml. of urine. Viscosity determinations were carried out immediately after addition of the urine to the buffer-substrate mixture and at the end of 20 minutes incubation. Viscosity reductions were expressed in seconds occurring over the 20 minute period. Controls consisted of an aliquot of the same urine sample previously heated for 40 to 50 minutes in a boiling water bath to destroy enzyme activity.

Three groups of experimental procedures were carried out.

Group I—*Relationship of urine osmolality and urinary hyaluronidase activity.*

Osmolalities and hyaluronidase activities were determined on urine samples from 15 subjects collected for a one-hour period one-and-a-half-hours following an intramuscular injection of 2 units of pitressin. Changes in viscosity over the 20 minute incubation period ranged from 1 second to 7.5 seconds. Arbitrary units of enzyme excreted per minute ranged from 6.5 to 63.0. The data suggested a direct relationship of urine osmolality to hyaluronidase activity but was not clear cut in all instances. For example two subjects concentrated to 1040 mOsm/kg. H_2O and one put out 63.0 units of enzyme/min. while the other subject excreted only 6.5 units.

Group II—*Relationship of urinary hyaluronidase activity and time after pitressin injection.*

Serial samples of urine were collected at 15, 45, 75, and 105 minutes after administration of 2 units of pitressin subcutaneously in 6 subjects. The data suggested that maximal enzyme output occurred in the first forty-five minutes after administration of pitressin and then fell off in the later samples even though the urine osmolality remained elevated.

Group III—*Relationship of urinary hyaluronidase activity and time after pitressin injection.*

Group III showed a more apparent relationship between urine hyaluronidase activity and time after pitressin in 7 *water loaded subjects.* Maximal enzyme activity in the urine occurred during the first fifteen to forty-five minutes after pitressin and again later samples showed a low activity. In most subjects, even though the osmolality of the first 15 minutes sample was low, there was a high enzyme activity which would indicate that hyaluronidase output would follow the injection of pitressin and precede the concentrating of the urine.

Hyaluronidase activity was also shown in two catheterized subjects to rule out the possibility of periurethral secretion of the viscosity reducing substance. Two units of pitressin in a boiled urine control had no viscosity reducing properties.

From the data it is apparent that a viscosity reducing substance is present in the urine to the greatest extent in the first 45 minutes following pitressin injection and that this substance is not necessarily related to urine osmolality or urine flow as originally shown by Ginetzinsky.

GENETIC TRANSMISSION IN PITRESSIN-RESISTANT DIABETES INSIPIDUS AND A SEX DIFFERENCE IN PITRESSIN SENSITIVITY

Salvatore J. Cantolino and Muriel K. Dyke

Studies of males with pitressin resistant diabetes insipidus show that they are completely unresponsive to exogenous pitressin when changes in urine concentration are measured, while the mothers of these patients have a partial response to pitressin. Familial aggregation of the patients with this disease suggests the segregation of a sex-linked mutant gene with the heterozygotic carrier females representing an intermediate phenotype. In normal persons such a locus must be occupied by normal (wild type) genes of which the male has one, the female two. This difference in gene dosage might be reflected in a difference in sensitivity to pitressin; that is, the normal female with two genes might be more strongly affected than the male who has but a single gene. To test this hypothesis the following experiments were carried out.

Twenty-one young normal adult females and 18 young adult males served as experimental subjects. Females were tested only at their mid-menstrual period. A marked diuresis was induced by the oral ingestion of 25 to 30 cc. of water per kg. of body weight in about 1 hour. In addition, an alcohol load in the form of whiskey was given to eliminate endogenous secretion of ADH. When a urine flow of 10 cc. per minute or greater had been established the subjects were given 0.1 milliunit of pitressin per kg. of body weight intravenously, using a size 25 needle. Voided urine samples were then collected at 5 minute intervals until a pre-pitressin level of diuresis had again been established. Maximal diuresis was maintained during this time by the ingestion of an equivalent amount of water following each voiding. The Fiske osmometer was used to measure urine osmolality.

The results can be briefly stated as follows.

Males showed a peak urine concentration 15 to 20 minutes after the administration of the intravenous pitressin with the concentration falling back to the pre-pitressin level in 30 to 45 minutes. Maximum male concentrations ranged from 100 to 300 milliosmol/kg. The mean value was 157 milliosmol/kg.

Females showed a peak concentration response about 20 minutes after pitressin but had a generally longer duration of effect. The range of maximum female concentrations was 100 to 600 milliosmol/kg. The mean value was 279 milliosmol/kg. When plotted, the spread of female values showed a definite bimodal distribution with two-thirds of the values being between approximately 100 and 250 milliosmol/kg. and one-third of the values between 400 and 600 milliosmol/kg.

Control studies which included the repeated testing of individual subjects and single ovum twins demonstrated the reproducibility of the test and the highly significant difference found between males and females.

These results fulfill the hypothesis of a dosage difference between males and females. An alternative explanation which can not be ruled out at this time might be based upon the obvious hormonal differences between the two sexes.

NEW CONCEPTS CONCERNING GALLSTONE FORMATION

FREDERICK K. MERKEL

The mechanism responsible for the formation of gallstones has remained ill defined, despite the work of many investigators. Investigation of the crystalline portion of most gallstones reveals them to be of mixed nature, containing quantities of cholesterol, bilirubin, and calcium in varying proportions. In addition, several studies have shown that most stones have a significant amount of protein content.

The studies concerning themselves with analysis of the chemical structure of gallstones, and the numerous bacteriologic investigations of stone formation have failed to contribute much in the way of a clear cut explanation of their pathogenesis hence it was decided to explore the microscopic character of these stones using the techniques of electron microscopy and histochemistry, which had not as yet been applied in this area.

Electron microscopic studies revealed the presence of a characteristic, filamentous interlacing matrix. This matrix was composed of a homogenous background substance in which are located numerous osmophilic granules.

Histochemical studies showed that this matrix reacts strongly to PAS stain indicating the presence of mucoprotein, and reacts with a brilliant metachromasia to toluidine blue, denoting the presence of mucopolysaccharide. The delicate, interlacing matrix is coated with a thin sheath of tightly bound pigment, most certainly direct bilirubin, which blocks the PAS but not the toluidine blue reaction at the point of attachment.

We believe that the factors responsible for the formation of human gallstones may well be related to changes which influence the production of the matrix of these calculi, such as changes in pH or ion concentration, prehaps a direct result of obstructive phenomena in the region of the sphincter of Oddi.

The metabolism of bilirubin has been studied by Najjar utilizing colorimetric methods. He noted that direct bilirubin, in the bloodstream, is chelated to serum albumin by means of manganese, magnesium, and to a lesser extent by copper and iron. When acted upon by the liver, the bond between the metal and the serum albumin is broken and the bilirubin passes into the biliary tree.

One could imagine quite easily that the bilirubin, along with its metal ion, when excreted into an obstructed biliary tree, could become chelated through its metal ion to the mucoprotein matrix, which has formed as a result of polymerization of smaller units, this being directly a product of common duct obstruction.

The direct bilirubin now chelated to the matrix would form the shell noted previously. With this structural framework present, cholesterol, bilirubin, and calcium would be easily deposited, resulting in a mixed stone such as is so frequently found in patients suffering from cholelithiasis.

THE EFFECTS OF HOST HORMONES ON THE GROWTH AND DEVELOPMENT OF *HYMENOLEPSIS DIMINUTA* IN RATS

G. RANDOLPH HAND

It was shown by Addis (1) that *H. diminuta* grown in gonadectomized male rats were on the average much shorter than normal worms, and that administration of testosterone to the castrated host maintained normal length. This project was de-

vised to reexamine the data of Addis with respect to earlier growth phases of the worms on the basis of length, weight, and proglottid number.

Four groups of 28 sexually mature white male rats were used in the experiments. Groups 1 and 3 served as normal controls and received a sham operation. The animals of Groups 2 and 4 were castrated. Throughout the experiment, each animal of Groups 3 and 4 was injected daily with 1 mg. of testosterone proprionate. The intermediate host used for *H. diminuta* was the flour beetle *Tribolium confusum*; after a two-week recovery period each rat received ten cysticercoids obtained from the beetles.

Four days following infection, ten rats in each group were killed and autopsied. All of the tapeworms recovered from each group of rats were pooled, and 25 or more removed and weighed. The rest were preserved and mounted whole on slides, and later the length and segment number of each was determined. After 8, 12, and 16 days, six rats in each group were killed, and the worms were removed and treated in the same manner.

Examination of the results shows that the worms of Group 1 become larger than those of Group 2 in terms of weight, length, and proglottid number as early as 12 days. The worms of Groups 3 and 4 are not significantly different in size, but they are larger at 12 days than the worms of either Group 1 or Group 2. At 16 days the worms of Group 1 seem to have caught up with those of Groups 3 and 4. Thus it appears that castration of the host does affect proglottidization of *H. diminuta*, and this effect seems to be prevented by providing the castrated host with testosterone.

Examination of the differential segment counts shows that at 12 days the worms of Groups 3 and 4 have more gravid segments than the worms of Groups 1 and 2. At 16 days, Group 2 lags significantly behind the other three in gravid segment count. Group 2 has the highest ratio of sterile to gravid segments and also has the lowest proportion of actively shedding worms in the sample. Thus it seems that castration of the host has also affected the process of maturation of the tapeworm, and this effect is similarly compensated for by the supplementation of testosterone.

In view of this evidence, it might be postulated that testosterone probably stimulates the growth and maturation of the tapeworm *H. diminuta*.

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THE STABILIZATION OF D-β-HYDROXYBUTYRIC DEHYDROGENASE. A NEW APPROACH TO THE OXIDATIVE PHOSPHORYLATION SYSTEM

James B. Wise

D-β-Hydroxybutyric dehydrogenase (BOH-D), first described 24 years ago (1), is the only DPN-linked dehydrogenase which has never been obtained in soluble form. In rat liver, it occurs exclusively in the mitochondrial membranes, associated with the respiratory chains. BOH-D catalyzes the reaction

$$\text{D-}\beta\text{-hydroxybutyrate} + \text{DPN}^+ \rightleftharpoons \text{Acetoacetate} + \text{DPNH} + \text{H}^+.$$

It can be assayed spectrophotometrically by following the absorption band of DPNH at 340 $m\mu$[2].

In the usual mitochondrial preparations BOH-D activity was quite labile. A more stable preparation, previously described (2), was washed mitochondrial membrane fragments prepared by disrupting mitochondria in a sonic oscillator, ultracentrifuging down the fragments, and washing these fragments through two more cycles. Stability was studied by incubating these fragments at 30°C. in dilute tris buffer, pH 7.4, and assaying aliquots at intervals.

Enzyme stability was greatest when the respiratory chains were kept fully reduced, as by hydroxybutyrate in the absence of oxygen, and was least when the chains were fully oxidized. Oxygen itself appeared to be the inactivating agent, as the particles were stable anaerobically, even in the presence of sufficient cytochrome C to oxidize all the respiratory carriers.

It was found possible to stabilize the enzyme in the presence of oxygen with a combination of three substances, namely succinate, DPN, and ATP, all of which were necessary for optimal effect. Succinate could not be replaced by other Krebs cycle acids, DPNH, or hydroxybutyrate. DPN could not be replaced by TPN or analogues. ADP had the opposite effect from ATP, preventing stabilization by DPN and succinate. In these washed fragments, the only enzymes present reactive with ADP and ATP are those of the oxidative phosphorylation system. The specific effects of succinate and ADP strongly imply an intimate association of BOH-D with succinic dehydrogenase and the oxidative phosphorylation system.

Chance and Hollunger (3) have reported that the endogenous pyridine nucleotides of intact mitochondria can be reduced by succinate if ATP is also provided, and they believe this to be a reversal of oxidative phosphorylation according to the net equation

$$\text{Succinate} + \text{DPN} + \text{nATP} \rightleftharpoons \text{Fumarate} + \text{DPNH} + \text{nADP} + \text{nP}_i .$$

In a much simpler system, these same three substances stabilize BOH-D.

It appears that BOH-D normally carries bound DPNH. If the DPNH is oxidized to DPN by respiration, it can be lost into the medium, and oxygen will then inactivate the exposed site. Succinate, DPN, and ATP are presumed to act together to keep the bound DPNH in its reduced form, possibly by reversal of oxidative phosphorylation.

Because of the apparent participation of phosphorylation in the above effects, these heavily sonicated particles were tested directly, and they were found to oxidize D-hydroxybutyrate with a P:O ratio of 1. Of the particles derived from mammalian mitochondria, these are perhaps the smallest and simplest ever shown to phosphorylate.

This work was done in collaboration with Dr. A. L. Lehninger.

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MECHANISM FOR D-AMINO ACID OXIDASE

Allen H. Neims

D-amino acid oxidase is a mammalian flavoenzyme which catalyzes the oxidative deamination of a wide variety of D-α-amino acids. Glycine oxidase, also a mammalian

flavoenzyme, catalyzes the oxidative deamination of glycine, an optically inactive, naturally occurring amino acid.

Both of these enzymes possess similar reaction pathways, cofactor, pH activity and substrate-structural requirements, sulfhydryl dependence, and solubility characteristics. The ability of D-amino acid oxidase to oxidize glycine is demonstrated by maintenance of a glycine/D-alanine specificity ratio of 0.03 (lamb kidney) and 0.015 (hog kidney) through a broad range of enzyme purification. The final hog kidney preparation possessed a specific activity equivalent to that of a recently reported crystalline enzyme (1). Hog kidney glycine oxidase (2) possessed the same glycine/D-alanine specificity ratio (0.015) as the purified D-amino acid oxidase. The oxidation of glycine by both presumed oxidases was markedly inhibited by equimolar concentrations of two substrate-competitive inhibitors of D-amino acid oxidase, L-leucine and cinnamic acid. Whether this pathway of glycine oxidation is of physiological significance remains to be demonstrated.

The substrate specificity of D-amino acid oxidase with respect to 16 phenyl-substituted phenylalanines and C-phenylglycines was investigated. Within the C-phenylglycine series, it appeared that the greater the electron-attracting capability of the substituted group, the greater was the substrate reactivity. This correlation was all but eliminated by the insulating β-carbon atom of the phenylalanines. Analysis of the reported Ki's of a group of substituted benzoates (3), substrate-competitive inhibitors of D-amino acid oxidase, revealed a similar dependence of inhibitor efficiency upon the electron-attracting capability of the phenyl substituent.

Electron-attracting phenyl substituents diminish electron density in the region of the α-carbon atom of both the C-phenylglycines and benzoates. Where an enzymatic nucleophyle, such as a sulfhydryl group, important in activating the substrate by attacking the α-carbon atom, a concept introduced with respect to pyridine-linked dehydrogenases (4), diminishing the α-carbon atom electron density would increase the frequency of such attack. Similarly, the inhibitor-enzyme complex would be stabilized.

This work was performed in collaboration with Dr. L. Hellerman and Dr. J. Wein.

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THE MEASUREMENT AND INTERPRETATION OF FREE TRANSLATIONAL DIFFUSION COEFFICIENTS OF BOVINE SERUM MERCAPTALBUMIN

Richard L. Hurwitz

Quantitative measurement of the translational diffusion process of a protein gives insight into the nature of protein-solvent and protein-protein interactions. The primary object of this work was to learn whether the superposition of a chemical reaction upon the diffusion process could produce changes in the diffusion coefficient that were predictable in terms of protein interactions.

Diffusion was carried out in a thermostated quartz cell. The progress of protein diffusion was recorded in the fringe pattern of a sensitive Rayleigh interferometer. By use of Fick's laws of diffusion and application of a mathematical method of Boltzmann, the diffusion coefficient of the protein may be determined as a function of the protein concentration. It is this functional relationship that permits conclusions to be made regarding protein interactions.

When salt free isoionic BMA is permitted to diffuse, from an initially sharp boundary, upward into a salt free aqueous layer, calculations show that the diffusion coefficient of the protein increases markedly as the protein concentration decreases. This observation is best explained by a specific interaction arising between hydrogen ion and protein. There is a direct correlation between the increase in the diffusion coefficient and the increasing dissociation of hydrogen ion from the protein as the protein concentration decreases.

When increasing quantities of KCl up to 5×10^{-4} M. are added to the solvent, the diffusion coefficient is seen to decrease progressively at the low protein concentrations—up to 0.1 per cent. At the higher protein concentrations—from 0.6 per cent to 0.9 per cent—such additions of salt increase the diffusion coefficient. The decrease can be explained in terms of a shielding effect of the salt, which acts to reduce the interaction between protein and hydrogen ion. The increase is a result of the binding of chloride ion to the protein. This binding affects protein-protein interactions in such a manner as to hasten the diffusion process.

In another experiment KNO_3 replaced KCl. Since more nitrate is bound per protein molecule than chloride under the same conditions, a further increase in the diffusion coefficient was expected at the higher protein concentrations. That the expected increase is observed gives support to the validity of the explanation put forth for the experiments with chloride.

The study of the diffusion of BMA under carefully controlled conditions reveals that protein interactions are a sensitive function of the protein concentration and of the composition of the solvent. Such studies may well become powerful tools in helping to unravel some of the many mysteries of protein behavior.

STUDIES ON THE PATHOGENESIS OF FEVER: THE DEMONSTRATION OF CIRCULATING ENDOGENOUS PYROGEN IN LEUCOPENIC RABBITS MADE FEBRILE WITH TYPHOID VACCINE

SAMUEL M. GILLMAN

The febrile response to intravenous injection of bacterial endotoxin has been thoroughly studied in relation to mechanisms involved in the pathogenesis of fever. The resulting response is characterized by a relatively long latent period of approximately 20 to 30 minutes, a prolonged febrile stage of 6 to 8 hours, and an immediate post-injection leucopenia followed by leucocytosis.

A second type of experimental fever, which results from the injection of saline extracts of polymorphonuclear leucocytes, is readily distinguishable from that caused by endotoxin. Its characteristics include a shorter latent period of about 10 minutes, a briefer febrile phase ending in a rapid defervescence, and a less pronounced post-injection leucopenia. Furthermore, if the leucocytic pyrogen is injected into endotoxin-tolerant animals, (animals made refractory by prior treatment with endotoxin), the febrile reaction is the same as in normal animals.

During endotoxin fever, there appears in the circulation an endogenous pyrogen,

the properties of which are indistinguishable from those of leucocytic pyrogen. Whereas most of the injected endotoxin is cleared from the blood stream during the latent period, the endogenous factor appears in the circulation at the onset of the fever and is present in a concentration which roughly parallels the degree of pyrexia.

The demonstration of endogenous pyrogen in the blood of animals with endotoxin fever has given rise to the hypothesis that the stimulus to the hypothalamic thermoregulatory centers which causes the fever is not the injected endotoxin itself, but rather the leucocytic pyrogen which is released from polymorphonuclear leucocytes injured by the endotoxin.

Despite a considerable body of experimental evidence which has accumulated in support of the endogenous pyrogen hypothesis, it has been challenged by a number of workers on the grounds that sera obtained during endotoxin fever from animals made leucopenic with nitrogen mustard have been said to contain no transferable pyrogen. These crucial nitrogen mustard experiments have been repeated and extended in the present study. The results which have been obtained indicate:

1) that rabbits made severely granulocytopenic with nitrogen mustard generated endogenous pyrogen when given a fever-producing dose of bacterial endotoxin;

2) that such rabbits produce less endogenous pyrogen than normal rabbits given the same endotoxic stimulus;

3) that the failure of previous investigators to detect endogenous pyrogen in the blood of febrile leucopenic animals has been due to the use of inadequate amounts of serum in the passive transfer test;

4) that the correlation between the number of circulating granulocytes and the amount of endogenous pyrogen produced strongly suggests that the principal source of endogenous pyrogen is the granulocytes; and

5) that the hypothesis is thus further strengthened that leucocytic pyrogen plays a central role in the pathogenesis of fever.

EXPERIMENTAL STUDIES OF *SALMONELLA* BACTERIEMIA

CLYDE L. OLSON

Intravenously injected bacteria are rapidly cleared from the bloodstream of the experimental animal. The rôle of the reticulo-endothelial system, certain natural and acquired blood factors, and more recently the rôle played by the leukocyte in clearance has been studied by others. The effect of dose on clearance of bacteria from the bloodstream of the experimental animal has not been well studied and the effect of immunity has been somewhat controversial. The purpose of these studies was to determine the effect of dose on the clearance of *S. typhi* (TY2) from the bloodstream of the normal, leukopenic and immune rabbit.

In normal animals, doses of 10^2 and 10^4 were completely cleared from the bloodstream within five minutes after intravenous injection and repeated blood cultures were negative. Doses of 10^5 and 10^6 were cleared within 10 minutes. When the dose was increased from 10^7 to 10^8, the initial rapid phase of clearance was followed within 30 minutes by a low level persistent bacteriemia which lasted about 24 hours. The effect of comparable doses on the nitrogen mustard-induced leukopenic rabbit was qualitatively similar to that seen in the normal rabbit although the former cleared fewer organisms in the first 10 minutes when doses of 10^7 and greater were used. The immune animal clearly removed more organisms per unit time than did either the normal or the leukopenic animals regardless of the dose given. Only with doses of 10^8 and 10^9 was the persistent bacteriemia noted. It lasted for about 24 hours.

Our studies indicate that the immune rabbit removes greater numbers of typhoid bacilli from the bloodstream than does the normal or leukopenic rabbit when comparable doses are injected. This difference is transient, however, for within 15 minutes after injection of doses of 10^7 to 10^9 organisms, each group of animals will have cleared in excess of 98 per cent of the inoculum. Secondly, although the rate of clearance decreases as one increases the dose, one finds when comparing dose given to per cent of inoculum cleared per unit time that increasing the dose does not retard clearance of the organism. Lastly, persistent low level bacteriemias were seen in the normal and leukopenic animals with doses of 10^7 and greater whereas only with doses of 10^8 and 10^9 did this phenomenon occur in the immune rabbit.

A STUDY OF SINGLE POLYNUCLEOTIDE CHAINS DERIVED FROM T_2 AND T_4 BACTERIOPHAGE

KENNETH I. BERNS

The mechanism of viral infection has received a great deal of attention in the last decade through the study of the bacteriophage-bacterium system. In 1952 Hershey and Chase showed that DNA was the infective material during bacteriophage T_2 infection of the bacterium *Escherichia coli*. Since then an extensive amount of work has been done on the genetics of bacteriophage. It is now assumed that DNA is in fact the genetic material. Therefore, it is of interest to be able to correlate the physical structure of DNA with the genetic results.

The Watson-Crick model of DNA assumes that the helix is made up of two continuous complementary polynucleotide chains held together by hydrogen bonding between complementary bases. During replication the two complementary chains separate and each serves as a template for the production of new DNA.

It is now believed that bacteriophage T_2 and T_4 DNA exists as one large molecule of DNA for each phage. If the Watson-Crick model for replication were correct, one would expect that two of the approximately 200 bacteriophage progeny resulting from a single infected bacterium would be hybrids (that is, one-half of their DNA would be of parental origin) and that the other 198 progeny would have completely new DNA. Current evidence indicates that this is not the case. It appears that the parental DNA is broken down during replication and spread at random among the progeny.

The possibility was suggested that some sort of non-DNA linkage was holding together the various parts of the overall DNA molecule. However, no evidence has been found for the presence of protein links. Large DNA molecules treated with proteolytic enzymes show no drop in molecular weight.

Another possibility was the presence of single polynucleotide chain breaks. Such breaks could exist in the large overall DNA molecule, which would then be held together by the hydrogen bonds between the two complementary chains. During replication, though, when the two strands separate, the DNA would break down into a large number of small fragments. Such a model would have been consistent with both the genetic evidence and the then available physicochemical evidence.

Therefore, the following experiment was performed in conjunction with Dr. C. A. Thomas, Jr. of the Homewood campus. By several methods of denaturation phage T_2 and T_4 DNA was separated into the individual polynucleotide chains from the original double helix. Denatured DNA was maintained as such by the addition of 1 per cent formaldehyde, which had no effect on the native material. Comparative molecular weights of the native, double helical material and the denatured single stranded material were then determined using the method of CsCl density centrifugation. If single

strand breaks were present, the molecular weight should have fallen by more than one-half. However, the molecular weight of the denatured material was in all cases one-half that of the native material; thus ruling out the possibility of single strand breaks. That we could determine a small number of single polynucleotide chain breaks was shown in a control using mild DNAase treatment.

STUDIES ON RESISTANCE TO 6-MERCAPTOPURINE IN LEUKEMIC CELLS

EDWARD I. RADEL

This project as conceived by Dr. Albert Owens, Jr. is designed for the purpose of developing a simple *in vitro* clinical test to be used for the study of resistance developed in leukemia patients to the administration of the antimetabolite, 6-mercaptopurine (6MP). It is hoped that such documentation of resistance will lend itself to intelligent management of these patients and serve as a guide for the development of similar tests with other antimetabolites. Our work, now in its initial stages, is based upon experimental data found in L1210 mouse leukemia by Davidson, (1) in which resistance to 6MP could be demonstrated as a 'loss' of the "inosinic pyrophosphorylase pathway" used in mice to form the 6MP ribotide. Earlier workers (2) have shown that 6MP, although not believed to be incorporated in nucleic acids as 6MP ribotide, does inhibit the *de novo* synthesis of adenylic acid, and competes with hypoxanthine for incorporation into nucleotides. Because of this action of 6MP it was thought by Owens that analysis of nucleotides as such could reveal information about drug-resistance. Our early studies have indicated the following information on the development of this clinical test: 1) white blood cells have been separated by a new method (3) from red blood cells, and work with recently produced Difco Phyto Hemagglutinin may in the future effect a more adequate separation; 2) incubation of these white cells have been discovered by Espenshade in this laboratory to be dependent upon controlled methods using a Warburg apparatus containing these cells, either 6MP or some *de novo* test substance, Mg^{++} nutrients including glutamine, buffer, antibiotics, etc.; 3) after incubation, a modification of the LePage-Heidelberger Method (4) has been successfully used in obtaining a solution of pure sodium ribonucleotides for chromatographic analysis on a one-dimensional ascending system with Whatman #3 paper; 4) an isopropanol/H_2O/NH_3 system has been used for separation of adenylic acid (AMP) from other constituents after 16 hours—acid-soluble fractions of purines were found impossible to be resolved in this system satisfactorily, and further work will have to be done on this mixture to gain resolution; 5) electrophoretic methods (5) did not aid in separation using the methodology described above.

Future studies using the method described, and other refinements we hope to make, will consist of correlating glycine-2-C^{14} uptake into the AMP fraction in patients treated with 6MP with the state of resistance of those patients to the drug, so that certain criteria of resistance from *this* point of view will be arrived at, and at the same time running coordinate experiments on the enzyme pathway described by correlation of clinical resistance with the amounts of labelled inosinic acid formed after addition of labelled hypoxanthine-8-C^{14} to the incubation mixture, the latter substance competing with 6MP for the enzyme system. Direct enzyme assays have been demonstrated to give erratic results, and substrate uptake which is too rapid to measure differentially (3).

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LACTIC DEHYDROGENASE PATTERNS IN THE TISSUES OF NORMAL AND TUMOR BEARING RATS

FREDERICK M. ROSENBLOOM

In recent years investigators have shown by various immunochemical and biochemical techniques that enzymes catalyzing the same reaction may exist in multiple molecular forms. Other evidence for the molecular heterogeneity of an enzyme has been provided by Markert (1) using the technique of zone electrophoresis on starch gel. Each form of the enzyme detected by this method is called an 'isozyme'. The application of this technique to various rat tissue homogenates along with more extensive studies by other investigators suggests that different tissues in an organism have characteristic lactic dehydrogenase (LDH) patterns.

Wroblewski (2) and others have shown that LDH activity is elevated in the plasma of animals with various experimental tumors and in some patients with malignant disease. It is logical to assume that the tumor tissue is the source of the LDH in the plasma. Another hypothesis is that the elevated LDH activity is the result of the wasting of tissues which often accompanies malignant states. To resolve these hypotheses it was felt that a qualitative description of the LDH in the plasma and tissues of tumor bearing rats would be helpful.

The experimental approach was 1) to compare the isozyme patterns of LDH in tissues of normal and tumor bearing rats; 2) to establish isozyme patterns of LDH in tumor tissues and 3) to serially follow isozyme patterns of LDH in the plasma of tumor bearing rats.

The LDH isozyme patterns of various rat tissues were studied. These results indicated that quantitative as well as qualitative differences determine the specificity of the isozyme pattern for each tissue. Identical patterns were found for the comparable tissues of normal and tumor bearing rats.

Two tumors were studied. One a spontaneous Walker tumor, the other a chemical tumor induced by the oral administration of methyl cholanthrene. Each tumor contained 2 isozymes which were noted to be electrophoretically identical to the corresponding isozymes found in other rat tissues. The patterns exhibited by the 2 tumors were the same and distinct from the pattern present in any normal rat tissue.

The evolution of plasma LDH was followed in rats implanted with the Walker tumor. No LDH activity could be detected in the plasma of normal rats. Seven days after implantation of the tumor faint bands appeared in the plasma at positions of isozymes I and II. At 14 days these same bands had increased in intensity with no other bands noted. It was evident that the plasma LDH bands were distributed in the same pattern as those found in the tumor tissue.

In summary these observations suggested 1) that various tissues of normal rats exhibit characteristic LDH isozyme patterns; 2) that the patterns exhibited by the tissues of normal rats are identical to those observed in similar tissues from tumor rats; 3) that 2

etiologically distinct tumors possess identical LDH isozyme patterns and 4) that the similarity between the LDH isozyme pattern of plasma and tumor from tumor bearing rats indicates that the tumor is the source of the enzyme in the plasma.

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IN VIVO AND *IN VITRO* DEMONSTRATION OF TUMOR IMMUNITY TO THE TRANSPLANTED MELANOMA OF HAMSTERS

Steven A. Rosenberg

In 1958 Fortner described a series of spontaneous, transplantable malignant melanomas in the Syrian hamster which exhibit striking similarity in histogenesis and behavior to the human counterpart of this tumor. Both melanotic and amelanotic strains were described (1). The pathologic and *in vitro* behavior of these tumors has recently been the subject of extensive studies (2, 3). This melanoma has been used to investigate the phenomenon of host immune reactions to the growth of transplanted tumor. Both *in vitro* and *in vivo* studies have been conducted.

Tumors were implanted subcutaneously and intramuscularly to the right femoral area of hamsters. Mean survival time of hamsters receiving the amelanotic strain is 38.7 $\pm$ 4.2 days (mean $\pm$ standard error). Mean survival time for the melanotic strain of tumor is 66.3 $\pm$ 7.4 days. After 3 to 5 weeks blood was withdrawn from the hamsters, serum was separated, and complement was destroyed by heating to 56°C. for 30 minutes. Serum from normal, healthy hamsters was collected in an identical manner. These sera were added to tissue cultures of growing melanoma cells obtained from other hamsters. The serum withdrawn from the tumor-bearing animals destroyed the cultures in from 1 to 9 days. Serum from the normal hamsters had no inhibitory effect on the growth of the cultures. The growth of the melanoma in the hamster thus results in the appearance of a factor in the serum of the hamster capable of destroying the melanoma cells when grown in tissue culture.

One hundred hamsters from 10th generation inbred strains (laboratory of Dr. J. C. Rosenberg, University of Minnesota Hospitals) were matched into pairs of comparable age, weight, and sex. One member of each pair was randomly selected to receive subcutaneous injections of the melanotic melanoma. Seventy-two per cent of the animals developed a tumor. After two months hamsters that did not develop the tumor (28%) and their previously untreated matched controls received implantations of tumor fragments to the left femoral area. Tumor implantation results in a slightly higher incidence of tumor 'take' than the tumor injection used in the first part of the experiment. A 25 per cent take was obtained in the group that had been previously sensitized. The control group demonstrated a 92 per cent take. This difference is significant to greater than the 99.9 per cent confidence level. Previous exposure and rejection of the melanoma by the hamster thus increases the resistance of the hamster to subsequent implantation of the melanoma.

Work on the specificity and precise identity of the factor involved is required. The experiments described point to the conclusion that the growth or exposure of the hamster to the melanoma results in what i thought to be an immune reaction capable

of either destroying the melanoma when grown in tissue culture or resisting the growth of the tumor in the intact animal.

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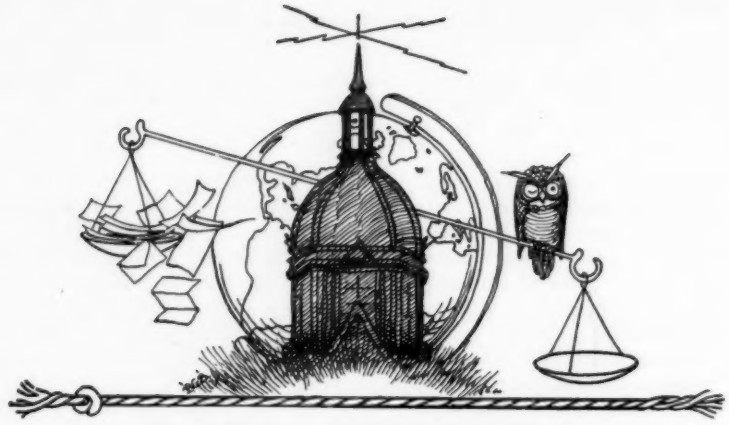

BOOK REVIEWS

Der Schnupfen, Beiträge zur Ätiologie, Pathogenese, Diagnostik und Therapie mit besonderer Berücksichtigung der Allergie, 2nd edition. By G. EIGLER, und D. G. R. FINDEISEN. Cloth. 12.00 marks. Johann Ambrosius Barth, Leipzig, East Germany.

The fact that this small volume, covering a variety of aspects of rhinitis or "rhinopathy", has required a second edition less than a year after its appearance is testimony to the interest in its subject. There are 13 chapters written by as many authors from West and East Germany, Czechoslovakia, Austria, Hungary, and The Netherlands. Inevitably, we find not only overlapping but also contradictory statements. As a result, it cannot serve as a guide to the student or young physician, but the book has its merits in pointing out unsolved problems and variety of opinions. After an introductory chapter on pathology and pathophysiology there follows a discussion of virus infections. Of special interest are the three chapters on histological and histochemical changes in the nasal mucosa; they present original work done with special staining methods and illustrated with excellent microphotographs. The other sections deal with problems of treating allergic rhinitis. Specific desensitization in Germany still has to rely on extracts made in England (p. 40). Hajos of Hungary believes that except for seasonal hayfever the allergic diseases of the respiratory tract are predominantly based on bacterial sensitization. Consequently, treatment is given with autogenous vaccine or tissue extract ("autolysates") in short courses which are repeated every six to twelve months. On the other hand, the chapter on surgery of polyps questions the value of desensitization, and, favoring combination of the Caldwell-Luc procedure with radical surgery of the ethmoids, ascribes its good effects to reestablishment of the nasal passages and to an "unspecific stimulus". In a chapter on therapy-resistant allergic rhinitis, physical-dietary measures are recommended by Findeisen whose allergy clinic is attached to the University Clinic for Physical Therapy in East Berlin. Chapters on diagnosis and therapy of upper respiratory diseases in the infant and small child, and on occupational diseases of the nose conclude the volume.

S. WALTER LANDAU

Cirrhosis of the Liver. By MARTIN S. KLECKNER, JR. Foreword by J. Arnold Bargen and Archie H. Baggenstoss. 729 pp., $24.50. Charles C Thomas, Springfield, Ill., 1960.

The decade of the 1950's revealed cirrhosis to be the 10th most important cause of death in the U.S.A. and a rebirth of interest in this disease has appeared. Much productive research in the cause and treatment of the complications of cirrhosis has been done. It is little wonder that the mass of recent literature has been only partially digested by the practitioners of medicine. Dr. Kleckner has produced this monograph for the "busy clinician" aimed at both a critical understanding of the pathology, etiology, and diagnosis of liver disease and effective treatment.

The early chapters are splendid blends of illustrations and clear concise text. The historical and introductory chapter is embellished with 17 photographs of the pioneers of liver disease. The chapter on morphogenesis of cirrhosis uses the excellent drawings of Elias and Popper and is clearly the most lucid description of this difficult concept that I have seen. The brief review of experimental cirrhosis is a good digest of a bibliography of 194 items. Classification is given detailed consideration and a clinical list of portal, postnecrotic and biliary cirrhosis, hemochromatosis, hepatolenticular degeneration and cirrhosis in infants is presented. Each of these diseases is discussed in separate chapters. In these the author includes an extensive personal experience that is well organized, illustrated and concisely set forth in comparison with standard literature statistics. Though these chapters do not reach the high level of the first ones of the book, the author is comfortable in this writing, well acquainted with the literature and his material. He writes in a readable clear fashion. Generous illustration is used to document or amplify most of the points in the text.

The chapter on cirrhosis in infants and the one on hepatic insufficiency are potpourri of great numbers of unrelated items, making organization and smooth reading impossible. Both chapters are documented with approximately 600 references and thus the items discussed and the detail considered must be arbitrary. The chapters on portal hypertension, ascites and hepatic insufficiency are *adequate* whereas previous portions of the work are excellent. Portal hypertension is properly detailed on diagnostic techniques. Balloon tamponade for bleeding varices is presented in detail, but the high incidence of complications and the hazard of aspiration is only distantly alluded to. The recent use of pharmacological agents in the alleviation of portal hypertension in bleeding varices is not mentioned. The pathogenesis of ascites is brilliantly presented, but under treatment there is no mention of the chlorothiazide type diuretics or aldosterone antagonists. The section on pathogenesis of liver coma and its treatment was the only one in the book with which the reviewer would disagree. Hyponatremia (p. 614) is presented as a significant cause of liver coma and this is documented by a case for which no data on Na is given. Hypokalemia, now widely accepted as important in some cases of hepatic coma is minimized. The use of wide spectrum antibotics in treatment is lumped with ACTH, steroids, glutamic acid, thioctic acid and arginine. A final chapter on the dietary treatment of cirrhosis is included containing charts that permit the clinician or his assistant to properly advise the patient without counsel of a professional dietician. The three page index is a gross injustice to the wealth of material contained in this book.

The printing is pleasing but a number of the illustrations are poorly reproduced so that careful study of the caption and the text must be made to determine what they are showing. Thus, the generally excellent illustrations are defiled by such poor ones as those on pages 150, 159, 359, and 601–4. The author occasionally illustrates a point with an extremely rare entity instead of a more representative example. Thus peliosis hepaticus

(p. 95), Gaucher's disease and cirrhosis (p. 149) and pericarditis with postnecrotic cirrhosis (p. 192) are shown. I believe that the publication date of September, 1960 is very misleading for though details of therapy include items available in 1958, many important concepts of 1959 have not been mentioned. Thus the Menghini biopsy needle, thiazide diuretics, spironolactone, and the particular value of the side-side portacaval shunt are not mentioned.

This book is a scholarly attainment of something new in the cirrhosis field. The synthesis of brief concise discussions of pathology, etiology, classification and diagnosis of cirrnosis is excellent and the inclusion of the personal series of the author is unique. This is the best single treatise on cirrhosis available and is admirably suitable for the clinically oriented doctor who needs an expert discussion of his problem. It is, therefore, highly recommended for all hospital and medical society libraries, so that all persons undertaking the management of cirrhotic patients will have access to it. It is hoped that a more current second edition will follow this one.

FRANK L. IBER

Surgical Diseases of the Chest. Edited by BRIAN BLADES. 280 pp., $22.00. The C. V. Mosby Co., St. Louis, Mo., 1961.

Thoracic surgery has expanded so rapidly in the last 15 years that concepts, principles, and practice have been changing and publication of a definitive text on thoracic surgery has been delayed. The breadth of the specialty and its separation along two slightly divergent lines, namely, cardiovascular and pulmonary, have almost precluded single authorship of an acceptable text. *Surgical Diseases of the Chest* is a compilation of the work of eighteen contributors covering in varying degrees of thoroughness all fields of thoracic surgery. The style, range, and depth of coverage vary from an almost too elementary one, as in the opening chapter on "Basic Physiology in Thoracic Surgery," to an up-to-date source of current information and references, as in the chapter on "Thoracic Aorta and Great Vessels."

The book is not the definitive work in a field which is still rapidly growing, but it helps to fill a gap which has been evident for some years, and will be particularly valuable for interns, house officers, and young surgeons. There are sure to be other books competing for eminence in this field in the near future, but this is at present a timely and valuable volume.

HENRY T. BAHNSON

A System of Medical Hypnosis. By AINSLIE MEARES. 484 pp., $10.00. W. B. Saunders Co., Philadelphia, Pa., 1960.

There are in English a variety of books available to the profession. A few are pioneer attempts to establish fundamental principles and practices by such innovators as Mesmer, Braid and Esdaile. There are also, the efforts of clinically oriented encyclopedists, represented by Moll, Bernheim and Bramwell. More recently with the revival of interest in hypnosis a number of texts have appeared, the best of which are by Brenman and Gill, Wolberg, Rosen, Schneck, and Marmer.

All of these current publications refer to the significance of "psychodynamics" and how necessary it is for the doctor, who uses hypnosis, to become acquainted with, and to master the principles of motivation, which are included in the term "psychodynamics". Mears' System of Medical Hypnosis, however, is the first book to effectively describe the nature and practice of what goes on between patient and doctor in the hypnotic inter-

relationship. It is the only book, which presents a succinct, practical outline of what is meant by the term psychodynamics, and how it is applied in the everyday practice of hypnosis.

The book is divided into five chapters: The nature of hypnosis; the induction of hypnosis; suggestive therapy; hypnoanalysis and hypnosis in general medicine.

The author has published six other books, has an "eclectic" interest in psychiatry and the "humbling experience" derived from an extensive practice of psychiatry. His text is a personal account in which he has succeeded in communicating his experiences with a variety of hypnotic techniques as he practices them. Dr. Mears also is an innovator and has introduced the use of painting (hypnography), modelling (hypnoplasty) and the Y (yoga) reaction. He reports the treatment of psychosomatic patients with asthma (good results), migraine (fair to poor results), skin conditions, anorexia nervosa and hypertension. There also are sections dealing with hypnosis in gynecology, obstetrics and as an anesthetic "agent".

Dr. Mears does not disparage "old fashioned" suggestive therapy, but rather indicates its value when properly used. He describes the technical aspect of hypnoanalysis in a concise, practical manner.

Probably the most valuable section for the physician and surgeon are the excellent descriptions of what occurs between doctor and patient before, during and after the induction of hypnosis. The reviewer has never read a better account of how the patient in the trance relationship communicates his needs to the doctor (pp. 28 to 38), the motivations for hypnosis, and the emotional relationship, which develops during the induction of hypnosis (pp. 125 to 135). The book contains an excellent glossary and index.

A System of Medical Hypnosis can be highly recommended as the best available text for any doctor who wants to learn something about the practical aspects of medical hypnosis

JACOB H. CONN

The Anonymous Mycobacteria in Human Disease. Edited by JOHN S. CHAPMAN. The Sixth Annual Ivan H. Mattson Memorial Conference of The University of Texas Southwestern Medical School and Dallas Tuberculosis Association. 173 pp., $7.50. Charles C Thomas, Springfield, Ill., 1960.

This is a timely and informative book on a subject of increasing importance. It brings together much data of extreme interest to any physician concerned with chest diseases. Among the participants of the conference reported here are several recognized authorities. Despite the title there is obviously a difference of opinion among the contributing authors as to whether "atypical" of "anonymous" is the preferable adjective.

The papers and discussions deal with bacteriology, pathology, clinical features and course, and therapy. Several conclusions appear permissible. Though the taxonomy is in a state of confusion a crude classification is possible. Fortunately, such grouping of organisms can be made usually satisfactorily and simply in a routine tuberculosis laboratory. Group I (photochromogens) and Group III (non-photochromogens) are the more common pathogens. Group II (skotochromogens) organisms are isolated most frequently but are less pathogenic. This group as well as the others may complicate various pathological conditions including pulmonary tuberculosis. There is no good basis for assuming these organisms are mutants of M. tuberculosis resulting from chemotherapy. Epidemiological studies indicate low infectivity. Though certain clinical features seem to appear more frequently with these infections than in those ascribable to M. tuberculosis, there

is no clinical diagnostic hallmark. Chemotherapy of such infections generally is not very satisfactory.

PHILIP F. WAGLEY

Essentials of Pharmacology, 4th edition. By FRANCIS K. OLDHAM, F. E. KELSEY and E. M. K. GEILING. 418 pp., $7.75. The J. B. Lippincott Co., Philadelphia, Pa., 1960.

The fourth edition of *Essentials of Pharmacology* keeps pace with the more recent developments in pharmacology by the addition of a new chapter entitled: "Hallucinogens and Tranquilizers." Sections on monoamine oxidase inhibitors and oral hypoglycemic agents have been added to the chapters on "Central Nervous System Stimulants" and "Endocrines" respectively. As in previous editions each chapter is concluded with a short but adequate bibilography and a list of important drugs, noting their official or non-official status. This book represents an introductory, up-to-date text which may serve the student of medicine, dentistry, veterinary medicine and pharmacy as an outline but should be used only in conjunction with more comprehensive texts. For schools of nursing *Essentials of Pharmacology* may be the best choice for a standard text. The authors themselves realize that the scope and the use of the book is confined. However, the reviewer feels that when the occasion calls for an introductory, and synoptic presentation of pharmacology this carefully written and concise text is an excellent choice.

G. I. KLINGMAN

Basic Psychiatric Concepts in Nursing. By CHARLES K. HOFLING and MADELEINE M. LEININGER. 540 pp., $6.25. J. B. Lippincott Co., Philadelphia, Pa., 1960.

The authors have combined their knowledge and experience in the field of psychiatry to present a well-organized and comprehensive reference-text. Emphasis is directed primarily toward application of principles in the psychiatric setting, but the reader is reminded throughout the book that patients are people, and that fulfillment of her professional responsibility demands that a nurse in any clinical setting understand concepts of human behavior and human relationships in order to deal effectively with less deviate expressions of emotional problems shown by patients. ". . . the aim . . . is not to make the student a specialist but more of a generalist. . . ."

Of particular value are chapters on nurse-patient relationships and the nurse's rôle in the various methods of psychiatric treatment, including somatic therapies; individual and group psychotherapies; milieu and activity therapies; there is frank and open discussion offered on the controversial issue of the nurse as a psychotherapist.

Table of contents, index, glossary and footnotes all contribute to excellent continuity. Bibliographies at the conclusions of each chapter are current and direct supplementary reading to the more valuable reference sources.

HARRY B. UPPERCUE

Meaning and Methods of Diagnosis of Clinical Psychiatry. By THOMAS A. LOFTUS. 169 pp., $5.00. Lea & Febiger, Philadelphia, Pa., 1960.

There is a real need for a short, clear, and concise book of this kind, coming as it does, at a time when many psychiatrists are reluctant to put a diagnostic label on their patients. The author, however, does not suggest that psychiatric diagnoses be purely descriptive in the Kraepelinian sense. He favors the use of Adolph Meyer's reaction types and in doing so implies that one has an obligation to understand the person who is doing the reacting and what he is reacting about. He emphasizes the need for a psychodynamic

understanding of the patient but cautions against the use of a jargon which would be understandable only to the initiated.

In reading hundreds of consultations by psychiatrists and other physicians throughout this country who have evaluated patients for Social Security disability determinations, the present reviewer has had a unique opportunity to observe the current usage of psychiatric diagnostic terminology. He would quickly agree with Eugen Bleuler who many years ago said, "with many patients the number of diagnoses made equals the number of institutions they have been to." The plain fact is that the word "physicians" could be substituted for the word "institutions" without altering the present day truth of the quotation. Such a lamentable situation might be largely corrected if Dr. Loftus' plea for diagnostic clarity and uniformity were taken to heart by those who put psychiatric labels on their patients. General practitioners and internists who surely make more of the total psychiatric diagnoses than the psychiatrists themselves would find this book particularly useful as would medical students and residents.

As in any book of this size, the necessity for over simplification may mislead the reader into believing that psychiatric diagnoses are more meaningful than they actually are. The Personal Diagnostic Formulation as outlined by Whitehorn and used at the Henry Phipps Psychiatric Clinic would supplement the formal diagnostic classification so well described by Loftus and lead to a better understanding of the personal meaning of the reaction by specifying the significant life situations and relevant personality characteristics. However, Loftus has made a valuable contribution toward clarifying some of the current diagnostic confusion in psychiatry.

JOSEPH H. STEPHENS

American Pharmacy. Textbook of Pharmaceutical Principles, Processes and Preparations, 5th edition. Edited by JOSEPH B. SPROWLS. 493 pp., $10.75. J. B. Lippincott Co., Philadelphia, Pa., 1960.

The 500-page revised Textbook of Pharmaceutical Principles, Procedures and Preparations is designed for the use of students beginning their training in pharmacy. It contains twenty-six chapters written by twenty contributors. The subtopics are presented in a concise and in most instances adequate manner; however, with a small number of subtopics the attempt at conciseness resulted in superficiality. In spite of this shortcoming the book will be useful to the pharmacy student as well as to students and scientists in the basic medical sciences who on occasion require an up-to-date reference book describing succinctly pharmaceutical principles, processes and preparations.

G. I. KLINGMAN

Synopsis of Pathology, 5th edition. By W. A. D. ANDERSON. 876 pp., $9.25. The C. V. Mosby Co., St. Louis, Mo.

It is a pleasure to review the fifth edition of Anderson's *Synopsis of Pathology*. In general the presentation is concise with emphasis placed on the more commonly encountered pathologic lesions. In addition, (1) certain more recent entities, such as catscratch disease and pulmonary alveolar proteinosis, and (2) the carcinoid syndrome, cytology, and mycotic infections are adequately emphasized. Photographs of gross specimens and photomicrographs are useful although some are poor in quality.

The bibliography might profitably have been increased, although it is evident that numerous recent references are cited. Additional discussions of recent contributions of experimental pathology (e.g., electron microscopy of blood vessels, immuno-fluorescence,

autoradiography and mechanisms of carcinogenesis and metastasis of tumors) would stimulate greater interest in the dynamics of normal and abnormal cellular behavior. For the instructor, a list of teaching motion picture films pertaining to fundamental pathologic processes, such as inflammation, thrombosis and neoplasia, would be a useful supplement.

This synopsis is warmly recommended for the student provided he employs it as a general review, not to replace, but to supplement the more extensive texts edited by Anderson or others.

SUMNER WOOD, JR.

BOOKS RECEIVED FOR REVIEW

Arthritis and Allied Conditions. A Textbook of Rheumatology, 6th edition. Edited by JOSEPH LEE HOLLANDER. 1306 pp., $20.00. Lea & Febiger, Philadelphia, Pa., 1960.

Atlas of Obstetric Technic. By J. ROBERT WILLSON. 304 pp., De Luxe Edition $14.50. The C. V. Mosby Co., St. Louis, Mo., 1961.

Control of Malnutrition in Man. 9th edition. Prepared by the Subcommittee on Control of Nutritional Diseases and authorized for publication by the Committee of Evaluation and Standards of the American Public Health Association. 140 pp. American Public Health Association, New York, N. Y., 1960.

The Hypothalamus of the Cat. A Cytoarchitectonic Atlas with Horsley-Clarke Coordinates. By RUTH BLEIER. 109 pp., $15.00. The Johns Hopkins Press, Baltimore, Md., 1961.

Key and Conwell's Management of Fractures, Dislocations and Sprains, 7th edition. 1153 pp., $27.00. The C. V. Mosby Co., St. Louis, Mo., 1961.

Managament of Hypertensive Diseases. By JOSEPH C. EDWARDS. With Foreword by Paul Dudley White. 439 pp., $15.00. The C. V. Mosby Co., St. Louis, Mo., 1960.

The Sex Technique in Marriage. Revised and Enlarged Edition. By ISABEL E. HUTTON. 191 pp., $3.00. Emerson Books, New York, N. Y., 1961.

Orthopaedia. Translated from the French of Nicolas Andry. A Photographic reproduction of the first English edition of 1743. Vol. I, 240 pp. Vol. II, 318 pp. $10.00. J. B. Lippincott Co., Philadelphia, Pa., 1961.

Some Reflections on Genius and Other Essays. By RUSSELL BRAIN. 192 pp., $6.00. J. B. Lippincott Co., Philadelphia, Pa., 1961.

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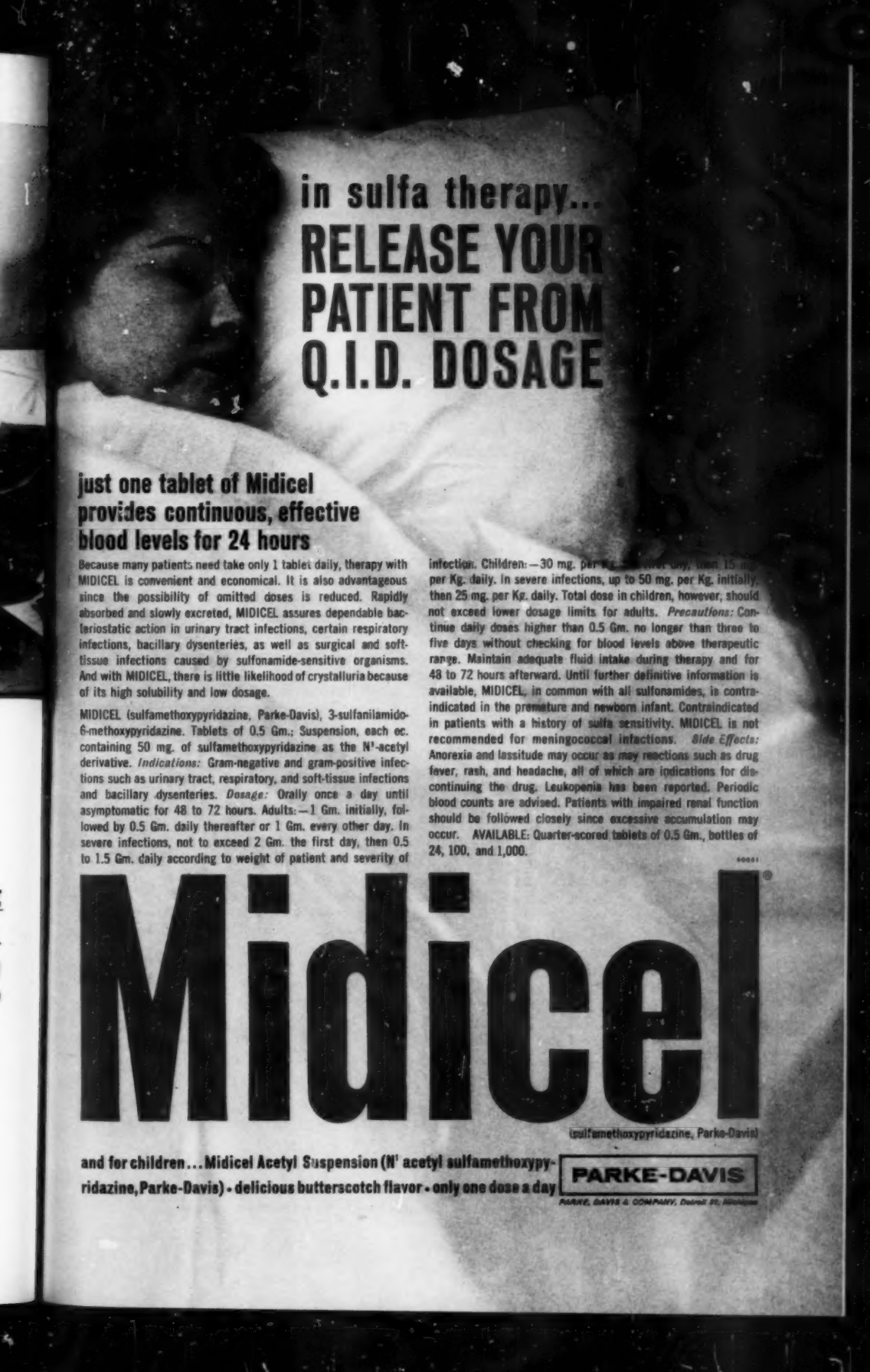

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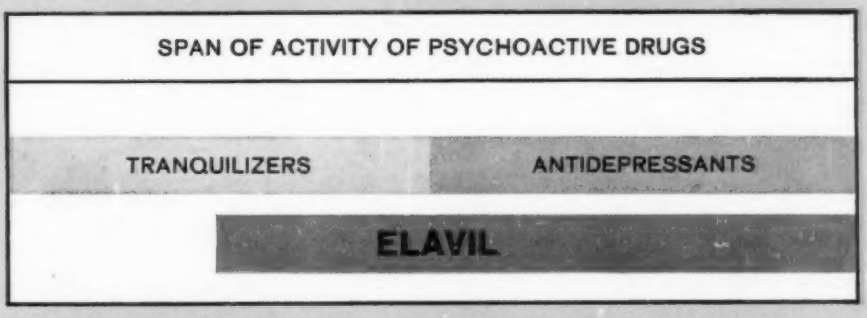
TRANQUILIZERS	"Failure of the tranquilizers to produce satisfactory results is due in many cases to their being prescribed for depression, especially depression masked by the more prominent symptoms of anxiety. The underlying depression may be deepened."[1]			+ −
ANTIDEPRESSANTS			"CNS stimulants and anti-depressants, if given to anxious patients, will increase the anxiety...."	+
ELAVIL	"... this drug [ELAVIL] acted both as a tranquilizer and as an anti-depressant..."[2] Many physicians customarily treat anxious or depressed patients with a combination of an antidepressant and a tranquilizer. This is seldom necessary when prescribing ELAVIL because it has both antidepressant and anti-anxiety properties.			++

ELAVIL

effective in patients with depression...
particularly useful in those with predominant symptoms of anxiety and tension...provides prompt relief of anxiety and insomnia associated with depression



INDICATIONS: Manic-depressive reaction – depressed phase; involutional melancholia; reactive depression; schizoaffective depressions; neurotic depressive reaction; and these target symptoms: anxiety; depressed mood; insomnia; psychomotor retardation; functional somatic complaints; loss of interest; feelings of guilt; anorexia. May be used whether the emotional difficulty is a manifestation of neurosis or psychosis,[4] and in ambulatory or hospitalized patients.[3, 4, 5]

USUAL ADULT ORAL DOSAGE: Initial, 25 mg. three times a day, until a satisfactory response is noted. Many patients improve rapidly, although some depressed patients may require four to six weeks of therapy before obtaining maximum benefit. In severely depressed patients, as much as 150 mg. per day may be given. **Maintenance,** 25 mg. two to four times a day. Some patients may be maintained on 10 mg. four times a day. The natural course of depression is often many months in duration. Accordingly, it is appropriate to continue maintenance therapy for at least three months after the patient has achieved satisfactory improvement in order to lessen the possibility of relapse, which may occur if the patient's depressive cycle is not complete. In the event of relapse, therapy with ELAVIL may be reinstituted.

ELAVIL is not a monoamine oxidase (MAO) inhibitor. No evidence of drug-induced jaundice or agranulocytosis has been noted. Side effects (drowsiness, dizziness, nausea, excitement, hypotension, fine tremor, jitteriness, headache, heartburn, anorexia, increased perspiration, and skin rash), when they occur, are usually mild. However, as with all new therapeutic agents, careful observation of patients is recommended. As with other drugs possessing significant anticholinergic activity, ELAVIL is contraindicated in patients with glaucoma.

SUPPLY: Tablets, 10 mg. and 25 mg., in bottles of 100. **Injection (intramuscular),** 10 mg. per cc., 10-cc. vials.

REFERENCES: 1. Perloff, M. M., and Levick, L. J.: Clinical Med. 7:2237, Nov. 1960. 2. Freed, H.: Am. J. Psychiat. 117:455, Nov. 1960. 3. Dorfman, W.: Psychosomatics 1:153, May-June 1960. 4. Ayd, F. J., Jr.: Psychosomatics 1:320, Nov.-Dec. 1960. 5. Barsa, J. A., and Saunders, J. C.: Am. J. Psychiat. 117:739, Feb. 1961.

Before prescribing or administering ELAVIL, the physician should consult the detailed information on use accompanying the package or available on request.

MSD MERCK SHARP & DOHME, DIVISION OF MERCK & CO., INC., WEST POINT, PA.

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THE CURRENT PLACE OF KANTREX (I. M.) IN THE SOLUTION OF A SERIOUS AND GROWING PROBLEM: RESISTANT GRAM-NEGATIVE INFECTIONS

A SERIOUS AND GROWING PROBLEM: An increasing number of reports[1-13] attest to the rising frequency of life-threatening infections due to Gram-negative organisms that are resistant to the commonly used antibiotics. Such infections have become almost as serious a clinical problem as those caused by resistant staphylococci. They assume critical importance in patients with low resistance and in patients compromised by debilitating illness.

HOW KANTREX (INTRAMUSCULAR) MEETS THE PROBLEM: Kantrex is effective against many strains of Gram-negative pathogens that are resistant to the commonly used antibiotics.[4,6,14-17] Kantrex is bactericidal, not merely bacteriostatic. It is rapidly absorbed after intramuscular injection, reaching peak blood levels in about one hour, and remains in most patients, in the active range for sensitive pathogens for 8 to 12 hours. Because Kantrex is bactericidal, it often produces a dramatic effect even in patients with lowered resistance. Of the few special-use antibiotics that have been found effective in combating resistant Gram-negative infections, Kantrex has the broadest history of clinical application.

AN UNSURPASSED SPECIAL-PURPOSE SPECTRUM: Kantrex is effective against a large number of resistant strains of the following organisms responsible for the great majority of life-threatening Gram-negative infections: E. coli, Aerobacter, Klebsiella, Proteus, and some strains of Pseudomonas. Kantrex is also active against Shigella and Salmonella. Response to Kantrex has been particularly gratifying in a large number of such infections that have been treated unsuccessfully with the other antibiotics. Because of its broad special-use spectrum and its bactericidal action, Kantrex has been suggested as the agent of choice in patients who clinically have Gram-negative bacteremia but for whom definitive bacteriological diagnosis is not yet available.[18]

CURRENT PLACE OF KANTREX AGAINST RESISTANT STAPHYLOCOCCI AND ENTEROCOCCI: In resistant staphylococcic infections, if for some reason Staphcillin may not be used, Kantrex should be considered among other antibiotics. In mixed infections due to strains of resistant staphylococci and Gram-negative organisms, both of which are sensitive to kanamycin, Kantrex may be a preferred antibiotic. Kantrex is also indicated in the treatment of some resistant enterococcic infections due to strains shown to be sensitive to kanamycin.

INDICATIONS: Kantrex is indicated in the treatment of Gram-negative, staphylococcic and enterococcic infections due to organisms shown to be sensitive to kanamycin, including strains resistant to other antibiotics. These infections include septicemia, postoperative wound infection, soft tissue infection, peritonitis, and pulmonary infection. Kantrex is of particular value in urinary tract infections due to Gram-negative organisms resistant to other antibiotics. Especially serious is a long-standing pyelonephritis in a debilitated patient, for such infections constitute the most common source of Gram-negative bacteremias[7,11,16] which may become life-threatening.

PRECAUTION: The major toxic effect of parenterally administered kanamycin is its action on the auditory portion of the eighth nerve. To minimize the risk of ototoxicity, the daily dose of 15 mg./kg. should not be exceeded nor should the duration of therapy be prolonged. The drug should be used with caution in reduced dosage in patients with impaired renal function to avoid excessively high blood levels. Evidence of renal irritation frequently occurs during therapy, but this effect appears to be reversible on cessation of therapy and is not necessarily an indication for withdrawing the drug if the patient's infection is responding.

There have been no reports of blood dyscrasias or other hematologic disorders, no liver toxicity.

TWO SPECIAL-PURPOSE ANTIBIOTICS: Bristol Laboratories provides antibiotics for the treatment of two major categories of resistant bacterial infection: Kantrex, for infections caused by Gram-negative micro-organisms resistant to commonly employed antibiotics; and Staphcillin, the unique new synthetic penicillin for the treatment of infections due to resistant staphylococci. Each offers the clinician an effective means of dealing with life-threatening situations in specific bacterial infections. Consult Official Package Circulars for complete information on dosage, administration and precautions for Kantrex and Staphcillin.

SUPPLY: Kantrex Injection, 0.5 Gm. kanamycin (as sulfate) in vial containing 2 ml. volume; and 1.0 Gm. in vial containing 3 ml. volume.

REFERENCES: 1. Finland, M.: *New England J. Med. 263*:207 (Aug. 4) 1960. 2. Cutts, M.: *Rhode Island M. J. 43*:388 (June) 1960. 3. Hannigan, C. A., *et al.: J. Maine M. A. 51*:77 (March) 1960. 4. Griffith, L. J., *et al.: Antibiotics & Chemother. 10*:88 (Feb.) 1960. 5. Editorial: *New England J. Med. 261*:1081 (Nov. 19) 1959. 6. Bernard, L. A. and Sutton, W. C.: *A.M.A. Arch. Int. Med. 105*:311 (Feb.) 1960. 7. Kirby, W. M. M. *et al.: J.A.M.A. 162*:1 (Sept.) 1956. 8. Bryer, M. S.: *Am. J. Med. 18*:782 (May) 1955. 9. Editorial: *New England J. Med. 262*:578 (Mar. 17) 1960. 10. Rogers, D. E.: *New England J. Med. 261*:677 (Oct. 1) 1959. 11. Holloway, W. J. and Scott, E. G.: *Delaware M. J. 32*:314 (Aug.) 1960. 12. Finland, M. *et al.: J.A.M.A. 170*:2188 (Aug. 29) 1959. 13. Schneierson, S. S.: *New York J. Med. 60*:3426 (Nov. 1) 1960. 14. Lattimer, J. K. *et al.: J.A.M.A. 170*:938 (June 20) 1960. 15. Holloway, W. J. *et al.: Delaware M. J. 32*:49 (Feb.) 1960. 16. Bunn, P. A. and Baltch, A.: *New England J. Med. 259*:659 (Oct. 2) 1958. 17. Koota, G. M. *et al.: New England J. Med. 263*:629 (Sept. 29) 1960. 18. Finegold, S. M.: *A.M.A. Arch. Int. Med. 104*:15 (July) 1959.

NOTE: Kantrex is also available in the form of capsules for local effect in gastrointestinal-tract infections, for preoperative bowel sterilization, and for adjunctive treatment in certain hepatic comas. The Capsule form is *not* intended for use in the treatment of systemic infections. See Official Package Circular for complete details.

KANTREX® (kanamycin sulfate injection)
A preferred antibiotic for Gram-negative infections resistant to commonly used antibiotics.

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BRISTOL LABORATORIES / Division of Bristol-Myers Co.
Syracuse, New York

SPECTRUM CHART OF BRISTOL ANTIBIOTICS

		KANTREX® (kanamycin sulfate)	TETREX® (tetracycline phosphate complex)	SYNCILLIN® (phenethicillin)	STAPHCILLIN® (sodium methicillin)
Gram-Pos. Bacteria	C. diphtheriae			•	
	Streptococci		•	•	
	Enterococci	•	•	•	
	Pneumococci		•	•	
	Staphylococci	•	•	•	•*
	Clostridium sp.		•		
Gram-Neg. Bacteria	Gonococci		•	•	
	Meningococci		•		
	H. influenzae		•		
	H. pertussis		•		
	K. pneumoniae	•	•		
	A. aerogenes	•	•		
	E. coli	•	•		
	B. proteus	•			
	Pseudomonas	•	•		
	Brucella		•		
	Salmonella	•	•		
	Shigella	•	•		
Rickettsia	R. akari		•		
	R. prowazekii		•		
	R. typhi		•		
	R. rickettsii		•		
	R. tsutsugamushi		•		
	R. burneti		•		
Viruses	Lymphogranuloma		•		
	Psittacosis		•		
Protozoa	E. histolytica		•		

*recommended for those staphylococci resistant to other penicillins

Since not all strains of the micro-organisms listed may be sensitive to a particular antibiotic, clinical therapy should be guided by *in vitro* antibiotic sensitivity tests whenever possible.

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in over six years of clinical use and more than 750 published clinical studies

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for relief of anxiety and tension

Outstandingly Safe

1 simple dosage schedule produces rapid, dependable tranquilization without unpredictable excitation

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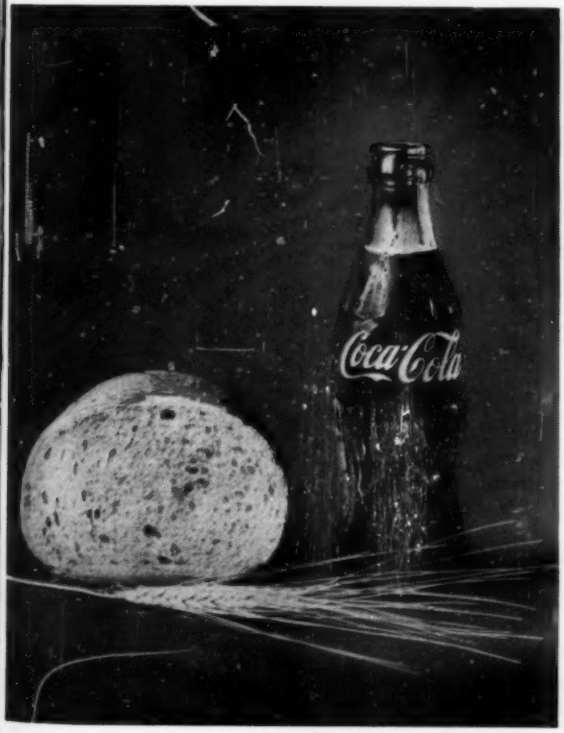

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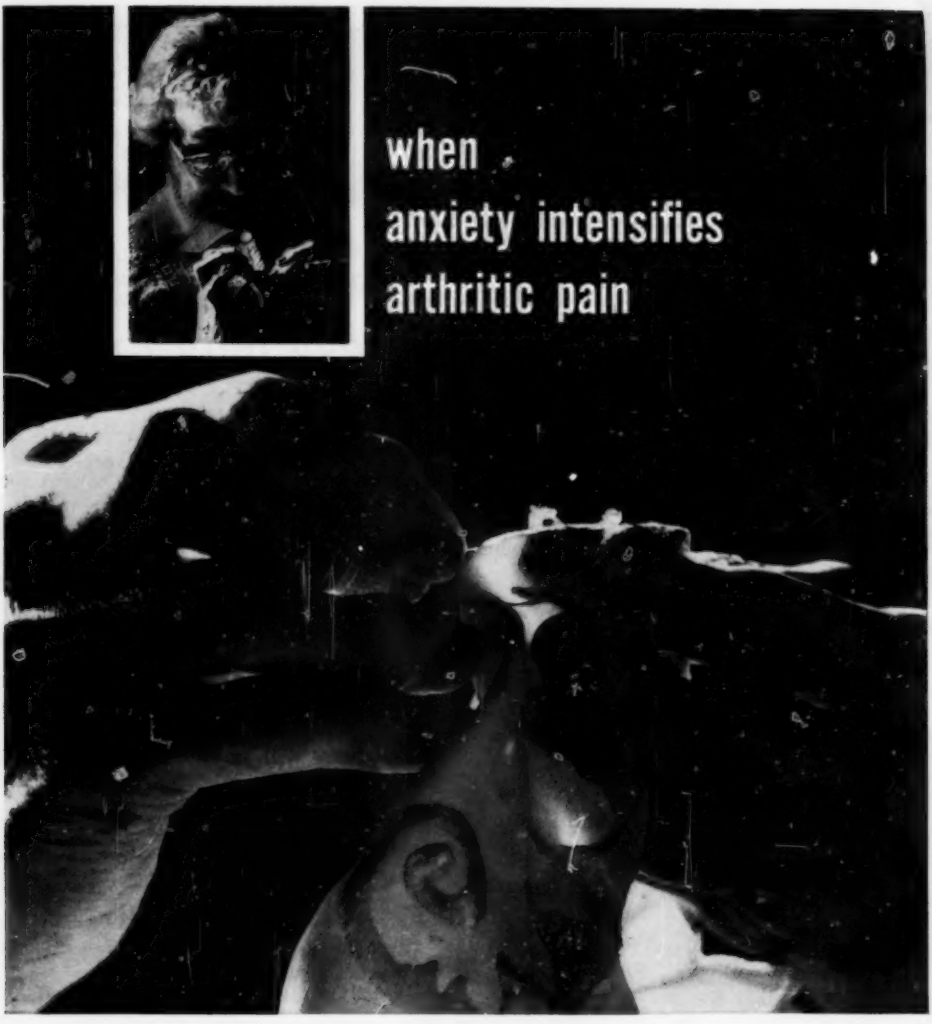

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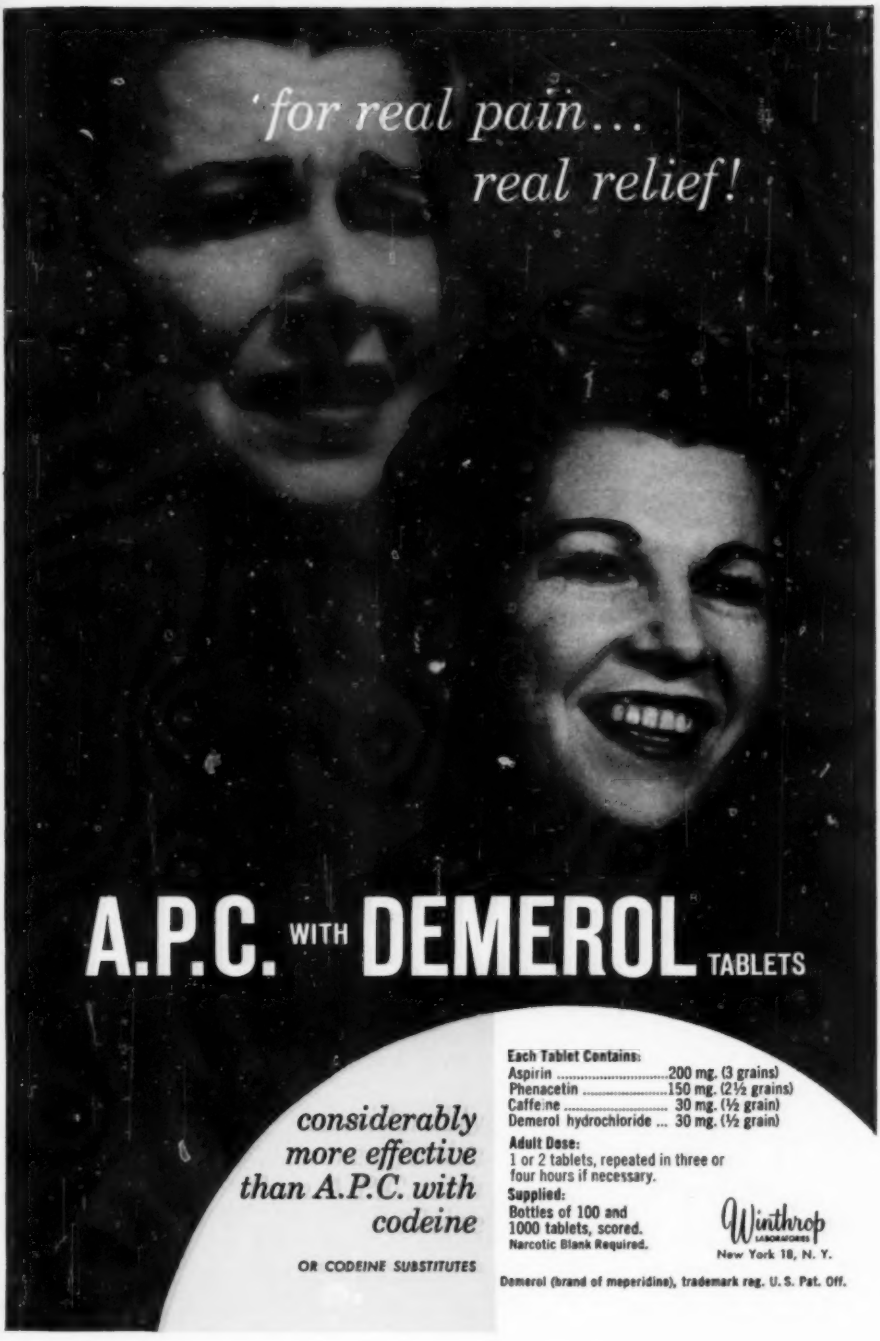
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